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# Effect of Antihistamine Levocetirizine Dihydrochloride on Cytogenetic Markers

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***Abstract***

*Levocetirizine dihydrochloride is the active ingredient of Alerinit, a second-generation antihistamine, used in the treatment of allergic diseases. This study was carried out to determine the probable genotoxic and cytotoxic effects of levocetirizine using chromosomal abnormality (CA) and micronucleus (MN) tests in human peripheral lymphocytes. In this study, cell cultures were treated with 2, 4, 8 and 16 µg / ml concentrations determined by preliminary study of Levocetirizine during 24 and 48 hours. As a result of our study, we observed that Levocetirizine does not cause any significant change compared to control in CA / Cell rate, abnormal cell percentage and mitotic index values, MN frequency, binuclear micronucleus cell rate and nuclear division index values at within the all studied concentrations and treatment periods. According to this research; levocetirizine dihydrochloride has no genotoxic and cytotoxic effects.*

***Keywords****: Chromosome Aberration, Cytotoxicity, Genotoxicity, Micronucleus, Levocetirizine Dihydrochloride*

## Introduction

Allergy is the hypersensitivity of the immune system to several substances called allergens. The concept of allergy was first described by the Austrian Clemens Von Pirquet in 1906 as “the body's response to foreign substances” (1). Allergic reactions have increased in developed countries due to the decrease in the diseases caused by infection and parasites, together with the environmental effects, but also the increase of individual hygiene and the lack of some

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immune system needed agents (2, 3). Antihistamines are drugs that bind to histamine receptors instead of histamine and block the receptors and thus eliminate the effects such as itching, and pain caused by histamine. Although these drugs are currently used as the first choice in the treatment of allergic diseases, H1 antihistamines are among all drugs are among the most widely used drugs (4, 5). There are different genotoxic studies on antihistamines. Since it does not significantly affect chromosome abnormality (CA) and micronucleus (MN) frequency in rats, fexofenadine has been reported to be not genotoxic (6). Terfenadine it was determined Chinese hamster that it did not affect MN frequency in V79 cells (7). Mizolastine, S. typhimurium has been reported to have no genotoxic effect in the presence and absence of liver enzyme complex in the Ames assays performed with strains TA98, TA100, TA1535, TA1537, as well as with E. coli WP2 uvrA (8). Cetirizine was not genotoxic in the Ames test system, CA in human lymphocytes *in vitro* and *in vivo* MN test systems in male rats (7). Astemizole did not induce SCE in human peripheral lymphocytes (9). Astemizole was not carcinogenic in long-term carcinogenicity testing with Wistar rats and Swiss rats (10). Roshdy (11) applied levocetirizine on pregnant mice and embryos during pregnancy to evaluate the active ingredient for cytotoxicity and mutagenicity. As a result, it has been reported that oral administration of levocetirizine at a recommended concentration during pregnancy can cross the placenta and cause mutagenic and cytotoxic effects on both mothers and embryos. Although this study was long-term, pregnant mice were regularly given an appropriate dose of levocetirizine daily. There is only one genotoxic study with levocetirizine, which is a frequently used antihistaminic drug group worldwide. More research should be done with this group of drugs on how antihistamines affect human genetic structure. Therefore, we determined the genotoxic and cytotoxic effects of levocetirizine in human peripheral lymphocytes by chromosome aberration and micronucleus tests.

**Material and Method**

The techniques of Evans (12) and Perry and Thomson (13) were followed for preparation of the CA test with minor modifications. This study was designed to follow IPCS guidelines (14). Whole blood (0.2 mL) from four healthy donors ( two male and two female, non-smokers, aged 22-25 years, blood samples not pooled) was added to 2.5 mL chromosome medium B ( Biochrom, F5023) supplemented with 10 µg/mL bromodeoxyuridine ( Sigma, B5002). The cultures were incubated at 37ºC for 72h, and then treated with 2, 4, 8, 16 µg/mL concentrations of levocetirizine dihydrochloride dissolved in distilled water, for 24h (levocetirizine dihydrochloride added 48h after initiating culture) and 48h (levocetirizine dihydrochloride added 24h after initiating culture). A negative control and a positive control (mitomycin, 0.1 µg/mL) were also used. Colchicine (0.06 µg/mL) was present for the last 2h of culture. At the end of the incubation, cells were harvested by centrifugation at 2000 rpm for 5min then the cells were treated with 0.4 % KCl as the hypotonic solution for 20 min and with fixative (methanol; glacial acetic acid 3:1) for 10 min at room temperature (22ºC±1, fixative treatments were repeated three times). The cell suspension was centrifuged at 1200 rpm for

10 min after each fixative treatment. After last fixative process, the cells were dropped on cold glass slides. After drying at room temperature for overnight, according to the standard method, the slides were stained using 5 % giemsa stain for CA test. MI was determined by scoring 3000 cells from each donor for each concentration. The percentage of cells, showing structural chromosome alteration, was obtained by calculating the percentage of the aberrant metaphases from each concentration and treatment period. CAs were evaluated in 100 well-spread metaphases per donor (totally 400 metaphases per concentration). CAs were classified as structural aberrations structural CAs were classified as chromatid and chromosome type abnormalities (breaks sister chromatid union, chromatid exchange, ring chromosome fragments and dicentric chromosomes). For the determination of genotoxicity, only the structural and numerical CAs were taken into consideration. For the analysis of Micronucleus (MN) binucleated lymphocytes, 0.2 mL of fresh whole blood (1/10 heparinized) was used to establish the cultures were incubated for 68h (15). The cells were treated with various concentration (2, 4, 8, 16 µg/mL) of levocetirizine dihydrochloride for 24h and 48h treatment periods. To block cytokinesis, cytochalasin-B (6 µg/mL) was added to culture 24h before the end of total incubation time. Finally, the cells were harvested by centrifugation at 2000 rpm for 5 min and process continued as mentioned above for preparation of CA slides, except for a 5 min hypotonic treatment at 37ºC. The cells were fixed once with cold fixative (1:glacial acetic acid, 5:methanol, 6:0.9%NaCl) for 20 min and the twice with second cold fixative (1:glacial acetic acid, 5:methanol) for 15 min after the fixation process, the cultured cells were centrifuged at 1200 rpm for 10 min. Finally, the slides were stained with 5 % giemsa. The number of micronucleated cells and total number of micronucleus present in 1000 binuclear cells (for each of the control and treated cultures) were determined. Also, a total of 1000 cells (4000 cells for each treatment concentration) were scored to calculate the nuclear division index (NDI) for the cytotoxicity of Levocetirizine dihydrochloride using the following equation (M1: mononucleated cells; M2: binucleated cells; M3 and M4: multinucleated cells and N is the total number of cells scored) (16). One-way ANOVA (Dunnet's test) was utilized for establishing the statistical significance of all parameters. CA, MN cells, MI, NDI data obtained from microscopic analysis were compared with control groups using SPSS (17.0) at $p \leq 0.05$. To find out the concentration response relation in treated groups, the regression analysis was performed.

## Results

In our study, we observed a slight increase in the frequency of abnormalities per cell compared to the control in all concentrations examined because of 24-hour treatment of levocetirizine in human peripheral lymphocytes. However, this increase was not statistically significant ($p > 0.05$). On the other hand, in human peripheral lymphocytes treated with levocetirizine at two concentrations (8 and 16 µg / ml) for 48 hours, a slight decrease was observed when the abnormal cell frequency was compared with the control, but this decrease was not statistically significant (Table 1) ($P > 0.05$).

Mitotic index (MI) value was determined by examining the effect of all doses of this active substance on mitosis because of treatment of human peripheral lymphocytes with levocetirizine for 24 and 48 hours. MI values were compared with the control group and levocetirizine caused a slight decrease in MI which was not statistically significant (p> 0.05). The effect of all doses of levocetirizine at 24 and 48 hours treatment time on MN frequency was compared with control; it was reported that this active substance caused a slight increase in MN frequency, but this increase was not statistically significant (P> 0.05).

**Table 1:** Abnormal cell and micronucleus finding in levocetirizine-treated human lymphocytes. MMC; Mitomycin C, PC; positive control, MN; micronucleus, SE; standard error.

| **Test Substance** | **Treatment** | | **Abnormal Cells (%) ± SE** | **MN (%) ± SE** |
|---|---|---|---|---|
| | **Duration (hr)** | **Conc. (µg/mL)** | | |
| **Control** | 24 | - | 3.50 ± 1.56 | 3.50±0.50 |
| **MMC (PC)** | 24 | 0.1 | 21.75 ± 2.15 | 23.75±2.75 |
| **Levocetirizine** | 24 | 2 | 5.25 ± 2.25 | 6.25 ± 2.06 |
| | 24 | 4 | 5.00 ± 1.78 | 6.00 ± 1.96 |
| | | 8 | 5.50 ± 2.60 | 6.005 ± 1.78 |
| | | 16 | 5.25 ± 2.36 | 8.75 ± 2.84 |
| **Control** | 48 | - | 3.50 ± 1.55 | 3.50 ± 0.50 |
| **MMC(PC)** | 48 | 0.1 | 40.40 ± 2.19 | 40.51 ± 2.12 |
| **Levocetirizine** | 48 | 2 | 4.00 ± 2.04 | 5.25 ± 0.85 |
| | 48 | 4 | 3.50 ± 1.44 | 5.75 ± 1.89 |
| | 48 | 8 | 3.25 ± 0.25 | 9.50±4.91 |
| | 48 | 16 | 2.00 ± 1.00 | 4.50±1.94 |

Furthermore, although all doses of levocetirizine active agent at both 24- and 48-hour treatment periods slightly increased binuclear micronucleus (BNMN), this increase was not statistically significant compared to control. (P> 0.05) Although all 24-hour doses of levocetirizine slightly reduced NDI compared to control, this reduction was not statistically significant (Table 2) (p> 0.05).

**Table 2:** Mitotic index (MI) and nuclear division index (NDI) findings in levocetirizine–treated lymphocytes. MMC; Mitomycin C, PC; positive control, SE; standard error.

| Test Substance | Treatment | | MI ± SE | NDI ± SE |
|---|---|---|---|---|
| | Duration (hr) | Conc. (µg/mL) | | |
| **Control** | 24 | - | 0.049 ± 0.017 | 1.074 ± 0.004 |
| **MMC (PC)** | 24 | 0.1 | 0.02 ± 0.01 | 0.1 ± 0.01 |
| **Levocetirizine** | 24 | 2 | 0.042 ± 0.018 | 1.051 ± 0.014 |
| | 24 | 4 | 0.032 ± 0.017 | 1.057 ± 0.010 |
| | | 8 | 0.040 ± 0.020 | 1.034 ± 0.009 |
| | | 16 | 0.036 ± 0.018 | 1.065 ± 0.011 |
| **Control** | 48 | - | 0.049 ± 0.017 | 1.074 ± 0.004 |
| **MMC(PC)** | 48 | 0.1 | 0.06 ± 0.01 | 0.2 ± 0.02 |
| **Levocetirizine** | 48 | 2 | 0.044 ± 0.013 | 1.078 ± 0.008 |
| | 48 | 4 | 0.037 ± 0.015 | 1.062 ± 0.007 |
| | 48 | 8 | 0.040 ± 0.017 | 1.075 ± 0.006 |
| | 48 | 16 | 0.043 ± 0.019 | 1.085 ± 0.004 |

## Discussion

In the present study, the genotoxic and cytotoxic effects of levocetirizine were investigated in human peripheral lymphocytes *in vitro*. In this study using chromosome aberration and micronucleus techniques, there was no induction in CAs and MN frequencies. Antihistamines have been used by patients for many years. The fact that a drug that should be taken regularly every day is not genotoxic is especially important for human health. Contrary to the genotoxic effect of many drugs in both community bias and previous studies, it is promising that levocetirizine does not have a genotoxic effect. Antihistamines have not been reported to have genotoxic, cytotoxic, or carcinogenic effects, not only in our study but also in many previous studies. Doxylamine has not been shown a mutagenic effect in Ames test. Zeiger et al. (17), Clemastine has been disclosed to affect micronucleus frequency in mice (7). Cyproheptadine has been reported to have no effect on CA value in human lymphocytes (18). In this study, negative results of doxylamine were obtained *in vitro* in human lymphocytes and in SCE test in fetal mouse cells (19). Methdilazine was negative in the Ames test with various strains of S.typhimurium in the presence and absence of S9 metabolic activation (20). Methdilazine has been reported not to induce CA in CHO cells (21). Triprolidine S. typhimurium TA97, TA98, TA100 and TA104 strains were reported to be non-mutagenic in the Ames test in the presence and absence of S9 fraction (22). Triprolidine has been reported to have no carcinogenic effect on long-term carcinogenicity testing in B6C3F1 mice and F344 rats (23). Chlorphenamine gene mutation test in mouse lymphoma L5178Y cells showed negative results (24). Chlorphenamine F344 in long-term carcinogenicity test with rats the results were negative (25). Promethazine has been reported to have no genotoxic effect in CHO cells (21). Promethazine was found not to induce CA in human leukocytes *in vitro*.

Gocke, (26). In Ames tests of Mizolastine with S.typhimurium TA98, TA100, TA1535, TA1537 and E.coli WP2 uvrA, there was no genotoxic effect in the presence and absence of liver enzyme complex (8). Terfenadine has been reported to not increase MN frequency *in vitro* in Chinese hamster V79 cells (7). Tripelennamine was not genotoxic in the gene mutation test in mouse lymphoma L5178Y cells (27). Astemizole has been reported to have no carcinogenic effect on Wistar rats and Swiss rats (10). It was reported that terfenadine did not increase the MN frequency *in vivo* and was not mutagenic in the Ames test with S. typhimurium strains (28).

As can observed, the lack of genotoxic effects of many antihistamines, especially levocetirizine, is promising, but there is only one genotoxic study of levocetirizine. Therefore, to have a clearer genotoxic effect of antihistamines, studies on these drugs are needed. Many studies using different techniques will support the clarification of the results.

## Conclusion

In the present study, it was shown that levocetirizine not induced the structural chromosomal aberrations, frequency of micronucleus and MI and NDI. It can be concluded that levocetirizine has not genotoxic and cytotoxic effects.

## Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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