INTRODUCTION

Dexchlorpheniramine is an alkylamine and a first-generation histamine antagonist with anti-allergic activity. Dexchlorpheniramine competitively blocks H1 receptors, thereby preventing histamine-induced effects on bronchial smooth muscles, capillaries, and gastrointestinal (GI) smooth muscles. The antagonistic action of dexchlorpheniramine blocks the activities of endogenous histamine, including histamine-induced bronchoconstriction, vasodilation, increased capillary permeability, and GI smooth muscle spasms. It is a classical, inexpensive drug with extensive clinical use, indicated mainly for allergic processes when histamine is the primary mediator (Calle, Fernandez-Benitez, 2004; Gobo-Oliveira et al., 2018). In cases of mild and chronic allergies, the dosage of the drug must be increased to avoid the development of drug tolerance. Dexchlorpheniramine is also a common ingredient in medicines for treating common cold. Several pharmaceutical dosage forms where dexchlorpheniramine is the single active agent or is present in association with other drugs can be found on the market. These associations typically contain active ingredients such as betamethasone, phenylephrine hydrochloride, and pseudoephedrine hydrochloride (Moreno et al., 2010). The most common adverse effects related to the use of dexchlorpheniramine are mild or moderate drowsiness (Boner et al., 1989), tiredness (Johansen, Bjerrum, Illum, 1987), low blood pressure, headache, and weakness and anaphylactic shock in rare cases.
Peripheral blood mononuclear cell (PBMC) isolation

Peripheral blood samples of three self-declared healthy individuals (two men and one woman, aged between 25 and 30 years) was collected in heparinized tubes by venipuncture (Ethics Committee of the Universidade Federal do Pampa, nº 27045614.0.0000.5323).

The pharmacokinetics of dexchlorpheniramine maleate was studied in healthy volunteers. Dexchlorpheniramine maleate had a half-life (t1/2) of around 20 hours (Li et al., 2008). The maximum concentration in plasma (Cmax) was 4.69 ng/mL and 4.92 ng/mL for dexchlorpheniramine maleate and the reference standard, respectively (Moreno et al., 2010).

Jacob et al. (2016) evaluated the acute toxicity of dexchlorpheniramine maleate in A. fischeri by measuring the luminescence of the bacteria after 30 minutes of contact with the drug. The authors reported that dexchlorpheniramine maleate in any formulation (generic, similar, or reference) did not exhibit toxicity in their experimental model. However, the effects of dexchlorpheniramine on healthy cells, especially on cell DNA, are yet to be understood.

Thus, the main objective of our study was to evaluate the effects of the dexchlorpheniramine reference standard (DCPA Ref. St) and a pharmaceutical formula on the DNA of human peripheral blood mononuclear cells (PBMCs).

MATERIAL AND METHODS

Chemicals

We purchased Sterile Histopaque 1.077, RPMI 1640 modified with 20 mM HEPES and L-glutamine, inactivated fetal bovine serum (FBS), phytohemagglutinin-M (PHA-M), penicillin/streptomycin, gentamicin solution, and dexchlorpheniramine maleate reference standard (DCPA Ref. St., Figure 1) (CAS number 2438-32-6) from Sigma Chemical Co. (St. Louis, MO, USA). The dexchlorpheniramine maleate pharmaceutical formula (DCPA) was obtained from a local pharmacy. Other reagents used in this study were of analytical grade and were stored according to the manufacturer’s instructions.

The PBMCs of each donor were separated by density gradient using Histopaque-1077®, united, and maintained in culture flasks containing RPMI 1640 supplemented with 20% FBS, 1% streptomycin/penicillin (v/v), and 0.2% gentamicin (v/v). The PBMCs were incubated overnight in a microenvironment at 37 °C with 5% CO2 levels to stabilize the cells. The cells were then stimulated

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**FIGURE 1** - Chemical structure of dexchlorpheniramine maleate. Empirical formula: C16H19ClN2O4; Molecular weight: 390.86.
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by adding PHA-M, and the cellular density was adjusted according to each assay protocol.

**Treatment schedule**

Five DCPA tablets were ground and weighed to calculate the average weight. The tablets contained 2 mg dexchlorpheniramine maleate, monohydrate lactose, starch, magnesium stearate, FD&C Yellow No. 6, Ponceau 4R dye, and gelatin. The DCPA Ref. St. and DCPA were dissolved in RPMI 1640 to final concentrations of 0.5, 2.5, 5, 10, and 50 µg/mL prior to use. The concentrations were selected based on the dexchlorpheniramine plasma peak and previous studies (Moreno et al., 2010; De Moura Leão et al., 2018). Each concentration test included three wells/flasks. The control group consisted of the solvent in the treatment medium (negative control, NC) and was processed in the same way as the cultures. Colchicine 10 µM was used as the positive control (PC).

**Trypan blue dye exclusion method**

Cell viability was assessed by the trypan blue assay, based on uptake of dye by unviable cells, which do not possess an intact membrane. Thus, the uptake of the dye causes the cells to appear blue stained. We plated the PBMCs at a density of 1 × 10^5 cells/well in sterile 24-well plates. Next, we exposed the cells to increasing concentrations of DCPA Ref. St. and DCPA for 24 h. Following the incubation period, the PBMC suspension was stained with 0.4% trypan blue (w/v) and counted under an optical microscope to estimate the number of live cells (Burow et al., 1998). Cell viability was expressed as the percentage of live cells in the NC.

**Determination of lymphocyte subpopulations**

We evaluated the specific toxicity of lymphocyte subpopulations, including CD3+, CD4+, and CD8+, using Countess II FL Counter (Thermo Fisher, Massachusetts, USA) equipped with EVOS™ Light Cube GFP fluorescence cube. We adjusted the PMBC density to 1 × 10^5/well and exposed the cells to increasing concentrations of DCPA Ref. St. and DCPA for 24 h. After this time-period in each culture, anti-CD3 (FITC), anti-CD4 (FITC), and anti-CD8 (FITC) antibodies were added, according to the manufacturer's specifications.

**Comet assay**

The comet assay was used to evaluate genotoxicity and was performed as described by Singh et al. (1988). For this, the PBMCs were cultivated in sterile 24-well plates at 1 × 10^5 cell/mL and exposed to different concentrations of DCPA Ref. St. and DCPA for 24 h. Subsequently, an aliquot of each culture was homogenized with low melting point agarose 0.75% (w/v), dispensed on slides pregelatinized with standard melting point agarose, and then covered with a coverslip until solidification. We immersed the slides in a lysis solution for 24 h. Electrophoresis was carried out (20 min at 300 mA and 25 V) in 300 mM NaOH/1 mM EDTA buffer, pH > 13. After alkaline denaturation, the slides were subjected to neutralizing solution, fixed, and stained with silver nitrate solution. The nucleoids were analyzed under an optical microscope at 400 × magnification and classified according to the damage levels with scores ranging from 0 (no migration of DNA) to 4 (maximum movement of DNA).

**Micronucleus test**

Mutagenicity was evaluated using the micronucleus test and performed according to the method described by Schmid (1975). For this, 1 × 10^5 PBMC/mL was seeded in sterile 24-well plates and exposed to increasing concentrations of DCPA Ref. St. and DCPA for 24 h. Then, we harvested aliquots from each culture for slide preparation, and subsequently stained them with fast staining kits. The aliquots were analyzed under an optical microscope at 1000 × magnification, and the PBMCs were scored according to the absence or presence of the micronuclei. The results are presented as the percentage of cells with micronuclei.

**Chromosomal aberrations test and mitotic index**

We conducted the chromosomal aberration assay as described by Yunis (1976) with modifications. For the assay, 1 × 10^5 PBMC/flask was stimulated with 1%
(v/v) PHA-M and incubated at 37 °C for 48 h. Next, we exposed the PBMCs to different concentrations of DCPA Ref. St. and DCPA for three hours. KaryoMAX Colcemid™ solution was added to the cultures for 3 hours before the harvesting period for cell cycle blockade. We centrifuged the cell cultures (1000 × g, 5 min), and the cell pellet was resuspended in 0.075 M KCl and again centrifuged (1000 × g, 5 min). The PBMCs were fixed with cold methanol:acetic acid (3:1) on pre-chilled microscopic slides. Subsequently, we stained the slides with 5% Giemsa and scored 300 well-spread metaphases for the presence of structural and numeric chromosomal alterations. Additionally, we calculated the mitotic index for each treatment as the number of dividing cells per 100 cells.

**Statistical analysis**

For all the assays, we verified the Gaussian distribution using the Kolmogorov–Smirnov test. Significant differences were determined using a one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test conducted using GraphPad Prism 7 software. The results were considered statistically significant at a p value <0.05.

**RESULTS AND DISCUSSION**

Dexchlorpheniramine maleate is a classical antihistamine widely used to treat allergic processes. However, its action is not selective, and it frequently inhibits the peripheral and central cholinergic receptors and serotoninergic receptors as well as affects other tissues (Calle, Fernandez-Benitez, 2004). In the present study, we evaluated the effects of DCPA, St. and DCPA on PBMC DNA.

We have provided experimental data on cell viability in Figure 2A. DCPA Ref. St. and DCPA did not induce cytotoxicity under the experimental conditions and the concentrations assayed in human PBMC culture when compared with the negative control. The cellular viability of all groups was approximately 98%. In addition to viability, we performed an experimental intervention with dexchlorpheniramine, which showed signs of toxicity in the CD3+ and CD4+ lymphocyte populations at a concentration of 50 ng/mL. We observed a reduction of approximately 62% in the CD3+ population. In CD4+ lymphocytes, the effects were worse, with a lymphotoxicity of 80% with the use of the dexchlorpheniramine maleate reference standard. The CD8+ population was not affected by dexchlorpheniramine (Figure 2B, 2C, and 2D, respectively).
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The CD3+ and CD4+ subpopulations are fundamental for proper functioning of the immune response system. The decrease in subpopulations when the highest concentration (50 ng/mL) of the DCPA. St. was used is worrying and could lead to an interruption in the body’s ability to respond correctly to an infectious process (Rich, Chaplin, 2019). Concentrations such as the one tested here (50 ng/mL) can lead to cases of overdose in patients with liver problems, leading to non-metabolism (Church, Church, 2013; Sanchez-Borges, Ansotegui, 2019). It can also affect renal function in patients with kidney problems and, consequently, obstruct the removal of the compound from the organism (Ricciardi, Furci, Stefania, 2019; Desai, Sehgal, 2020).

However, 150, 300, and 450 ng/mL of fexofenadine, a second-generation antihistamine, did not interfere with PBMC viability after 24 h of exposure (Kordulewska et al., 2015). Kontaş and Atlı Şekeroğlu (2015) found that loratadine is cytotoxic at concentrations > 1 µg. Thus, we believe that even though several antihistamines have the same mechanism of action and exhibit structural similarity, their toxic effects depend on frequency and exposure time. In addition, the different effects may be related to the different linking groups.

In our study, dexchlorpheniramine neither induced genotoxic or mutagenic effects nor numerical or structural chromosomal alterations, as can be seen in Figures 3, 4, and Table I. On the contrary, Kontaş and Atlı Şekeroğlu

FIGURE 2 - Effects of the dexchlorpheniramine maleate reference standard and dexchlorpheniramine maleate on peripheral blood mononuclear cell viability (A) and CD3+ (B), CD4+ (C), CD8+ (D) lymphocytes subpopulations. Data are expressed as mean ± standard deviation, n=3; experiments were performed in triplicates, and the results were analyzed by ANOVA followed by Tukey’s post hoc test. Significance was set at p <0.05. Different letters indicate statistically different values. NC = Negative Control (RPMI Medium); PC = Positive Control (Colchicine 10 µM).
Pamella Eduardha Espindola Chaves, Luísa Zuravski, Anelise Santos Soares

(2015) reported that 5, 15, and 25 µg/mL of loratadine increased the micronucleus frequency and chromosomal aberrations in lymphocytes after 48 h of exposure. It is essential to mention that, although dexchlorpheniramine and loratadine have the same mechanism of action, they do not necessarily have the same effects in these cells.

**FIGURE 3** - DNA damage index (A) and micronucleus frequency (B) in human peripheral blood mononuclear cells exposed to different concentrations of the dexchlorpheniramine maleate reference standard and dexchlorpheniramine maleate. Data are expressed as the mean ± standard deviation; n=3, experiments were performed in triplicates, and the results were analyzed by ANOVA followed by Tukey's post hoc test. The results were considered significant at p <0.05. Different letters denote statistically different values. NC = Negative Control (RPMI Medium); PC = Positive Control (Colchicine 10 µM).

**TABLE 1** - Chromosome analysis of PBMC after exposure to Dexchlorpheniramine maleate reference standard and Dexchlorpheniramine maleate

| Group                        | Mitotic Index (Medium ± SD) | Total metaphases | Normal metaphases | Numerical alteration | Structural alteration | Type of alteration |
|------------------------------|------------------------------|-------------------|-------------------|----------------------|----------------------|-------------------|
| **Negative Control**         |                              |                   |                   |                      |                      |                   |
|                              | 1.5 ± 0.57a                  | 300               | 300               | 0                    | 0                    | ------------------|
| **DEXCHLORPHENIRAMINE MALEATE REFERENCE STANDARD** |                              |                   |                   |                      |                      |                   |
| DCPA Ref. St. 50 ng/mL       | 1.25 ± 0.50a                 | 300               | 300               | 0                    | 0                    | ------------------|
| DCPA Ref. St. 10 ng/mL       | 1.75 ± 0.50a                 | 300               | 300               | 0                    | 0                    | ------------------|
| DCPA Ref. St. 5 ng/mL        | 1.5 ± 0.57a                  | 300               | 300               | 0                    | 0                    | ------------------|
| DCPA Ref. St. 2.5 ng/mL      | 1.5 ± 0.57a                  | 300               | 300               | 0                    | 0                    | ------------------|
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**TABLE I - Chromosome analysis of PBMC after exposure to Dexchlorpheniramine maleate reference standard and Dexchlorpheniramine maleate**

| Group            | Mitotic Index (Medium ± SD) | Total metaphases | Normal metaphases | Numerical alteration | Structural alteration | Type of alteration |
|------------------|-----------------------------|------------------|-------------------|----------------------|-----------------------|--------------------|
| DCPA Ref. St. 0.5 ng/mL | 1.25 ± 0.50 a               | 300              | 300               | 0                    | 0                     | ------------------|
| DEXCHLORPHENIRAMINE MALEATE | 1.75 ± 0.50 a               | 300              | 300               | 0                    | 0                     | ------------------|
| DCPA 50 ng/mL    | 2.00 ± 0.82 a               | 300              | 300               | 0                    | 0                     | ------------------|
| DCPA 10 ng/mL    | 1.5 ± 0.57 a                | 300              | 300               | 0                    | 0                     | ------------------|
| DCPA 5 ng/mL     | 1.75 ± 0.50 a               | 300              | 300               | 0                    | 0                     | ------------------|
| DCPA 2.5 ng/mL   | 1.5 ± 0.57 a                | 300              | 300               | 0                    | 0                     | ------------------|
| DCPA 0.5 ng/mL   | 1.5 ± 0.57 a                | 300              | 300               | 0                    | 0                     | ------------------|

DCPA Ref. St. = Dexchlorpheniramine maleate reference standard; DCPA: Dexchlorpheniramine maleate. The same letters in the column mean statistically the same values.

An essential factor to be analyzed is the risk of mutagenicity of the drugs. We performed this assessment using the chromosomal instability test (numerical and structural) and by calculating the mitotic index. The results are compiled in Table I and Figure 4. We did not observe numerical or structural alterations; no interference in cellular division was observed after exposure to the referred compounds.

**FIGURE 4 -** Representative images of the chromosome analysis of peripheral blood mononuclear cells before exposure (A: Negative Control) and after exposure to dexchlorpheniramine maleate reference standard (B) and dexchlorpheniramine maleate (C) at the highest concentration (50 ng/mL). The images were taken at 400X.
The results of the present study indicate the absence of mutagenic risk for the tested concentrations of both the pharmaceutical formula and the reference standard. Therefore, dexchlorpheniramine in both formulations does not induce DNA damage and genomic instability at the specified concentrations under the conditions detailed. However, in specific clinical conditions that cause an increase in plasma concentrations, it can lead to immunotoxicity of CD3+ and CD4+ subpopulations. Dexchlorpheniramine use should be reconsidered in clinical settings involving patients with liver disease or kidney failure of varying degrees.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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