Original Article

Study of Chlorpheniramine-induced Genotoxicity in Human Peripheral Blood Lymphocytes

Ehsan Zamani[1], Saba Mahboubi[2], Mehdi Evazalipour[3*]

1. Department of Pharmacology and Toxicology, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran.
2. Student Research Committee, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran.
3. Department of Pharmaceutical Biotechnology, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran.

*** Corresponding Author:**
Mehdi Evazalipour, PhD.
Address: Department of Pharmaceutical Biotechnology, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran.
Phone: +98 (13) 33486470-4
E-mail: evazalipour@gums.ac.ir, evazalipour@yahoo.com

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ABSTRACT

Background: Chlorpheniramine is an H_1 receptor inverse agonist, which belongs to the first-generation class. It is generally regarded as a strong antihistamine with a wide variety of indications in allergic and non-allergic diseases. The extensive consumption of chlorpheniramine might culminate in less evident adverse effects, such as genotoxicity.

Objectives: In this study, we attempted to assess the possible potential of chlorpheniramine in inducing genotoxicity.

Methods: Human lymphocytes were separated into groups as follows: control group (Phosphate Buffered saline), Chlorpheniramine group (0.1, 0.5, 0.75, 1.5 mM), and Positive control group (cisplatin 0.4 µg/mL). After 24 hours of incubation, we conducted an alkaline comet assay to evaluate the DNA damage. Also, oxidative stress damage was evaluated by the levels of lipid peroxidation and glutathione oxidation.

Results: Significant increases were observed in DNA percentage in tail and tail moment at high concentration (1.5mM, $P<0.05$). Likewise, at the same concentration, the MDA levels increased significantly in addition to the significant depletion in the level of glutathione.

Conclusion: High concentration of chlorpheniramine significantly induced genotoxicity in human lymphocytes. In addition, we showed that oxidative stress was one of the mechanisms elaborated in chlorpheniramine genotoxicity at high concentrations.

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Introduction

Antihistamine drugs are commonly referred to as a group of drugs, which mainly act as histamine 1 (H_1) receptors inverse agonists [1]. They have been widely prescribed since the 1940s in order to alleviate the symptoms of several allergic and non-allergic diseases [2-4]. The results of positron emission tomography on the first-generation antihistamines have revealed that these drugs can occupy virtually three-quarters of central H_1 receptors, which can result in sedation, fatigue, and performance and cognitive impairment [5, 6]. However, the binding to the H_1 receptors does not occur selectively. It has been demonstrated that these drugs have the affinity to inhibit cholinergic, and occasionally alpha-adrenergic and serotonergic transmissions [1]. Therefore, prescribing first-generation antihistamines might be followed by numerous side effects, including dry mouth, tachycardia, urinary retention, impaired movement, blurred vision, postural hypotension, increased appetite, and weight gain [1, 7-9].

Chlorpheniramine is regarded as a potent first-generation antihistamine with a short duration of action [10]. In terms of structure, it is classified into the alkylamine class [1]. In addition to the general indications of antihistamines, several surveys have shown that chlorpheniramine is practical in the prophylaxis and management of infusion-related reactions, in particular when administered concomitantly with a corticosteroid [11-13]. Apart from the common side effects, chlorpheniramine could possibly induce detrimental liver injuries through oxidative stress [14]. Besides, it is speculated that chlorpheniramine might have the potential to cause damage to the DNA [15, 16].

Oxidative stress is claimed to be a situation when the over-production of free radicals, especially reactive oxygen species (ROS), overpowers the antioxidant defense system [17]. This imbalance in the physiological system can ultimately have deleterious effects on macromolecules, such as nucleic acids, proteins, and lipids as well as organelles [18]. Particularly, several studies have provided further evidence for the genetic damages induced by oxidative stress [19, 20]. For instance, it is demonstrated in various studies that cisplatin can potentiate genotoxicity mainly through the promotion of ROS and inducing oxidative stress [21-23]. Thus, cisplatin is used as a positive control when the assessment of oxidative stress is the main objective of a study [24].

As regards the wide range of clinical indications of chlorpheniramine, it is noteworthy to assess its toxic features that are more ambiguous. Therefore, the main aim of this study was to assess the possibility of genotoxicity induced by chlorpheniramine in human peripheral lymphocytes and the role of oxidative stress in this event.

Materials and methods

Chemicals

Chlorpheniramine, thiobarbituric acid, Tris-HCl, tris ammonium, $MgCl_2$, disodium hydrogen phosphate, trichloroacetic acid (TCA), sucrose, ethylenediaminetetraacetic acid (EDTA), sodium acetate, and Triton X-100 were from Sigma; Dulbecco's modified eagle medium (DMEM) cell culture and phosphate-buffered saline (PBS) were from Gibco; phosphoric acid, potassium chloride, n-butanol, sodium chloride, 5,5-dithiol-bis-(2-nitrobenzoic acid) (DTNB), Na_2EDTA, sodium hydroxide, sodium lauroyl sarcosinate, dimethyl sulfoxide (DMSO) and Normal Melting Point (NMP) agarose were from Merck; and Low Melting Point (LMP) agarose was from Cleaver Scientific.

Blood sampling and treatment

The heparinized blood sample was collected from a young (24 years old) and healthy male donor (non-smoker and non-alcoholic,). The subject had not been exposed to any ionizing radiations or chemicals, which might have intervened in the results of this study, for six months in advance of the sampling time. The donor gave an informed consent form. The ethics committee of the Guilan University of Medical Sciences approved this study (Code: IR.GUMS.REC.1400.555).

The blood sample was mixed with DMEM, which was supplemented with 10% FBS, glutamax, and pen/strep. All experiments were conducted on the same blood sample. The resulting blood mixture was divided into separate groups as follows: control group (PBS), chlorpheniramine group (0.1, 0.5, 0.75, 1.5 milliMolar) [15], and positive control group (cisplatin 0.4 µg/mL) [25]. Then, for 24 hours, all samples were incubated (37°C and 5% CO_2 pressure).

Single-cell gel electrophoresis assay (alkaline comet assay)

The alkaline comet assay was conducted as described by Singh et al. with minor modifications [26]. Firstly, fully frosted slides were coated with NMP agarose.

Then, the samples were centrifuged at 200×g. Next, the pellet of each sample group was mixed with 1% LMP agarose and layered on the already pre-coated slides. Right after that, the slides were completely covered and put in a dark place at 4°C for 10 minutes so that the LMP agarose would solidify. After that, for 24 hours, the slides were placed in the lysis solution in a dark place and at 4°C. Subsequently, the samples were put in an alkaline electrophoresis buffer in a horizontal manner at 4°C for 20 minutes, and immediately after that, the electrophoresis procedure was conducted for 20 minutes at 4°C, 1V/m, and 300 mA. Tris buffer was used as a neutralizing agent, through which the slides were rinsed, and then, dehydration was done using ethanol. This stage was repeated two more times. Eventually, the slides were stained with SYBR® gold in dark for 15 minutes and rinsed with deionizing water. Totally, 100 nucleotides in each slide were assessed using an epifluorescence microscope at 200X magnification. The head and tail intensity were measured with CASPLab® (CASP1.2.3 beta2) software to quantify the DNA damage. At last, the tail moment was calculated.

Measurement of oxidative stress parameters

Measurement of lipid peroxidation

Malondialdehyde (MDA) levels were quantified using thiobarbituric acid as it was previously stated with minor modifications [27]. Cell homogenate pertaining to each group was first mixed with 0.05M sulfuric acid followed by the addition of 0.2% TBA and subsequently, the samples were put in boiling water for 30 minutes. Immediately after that, the samples were placed in an

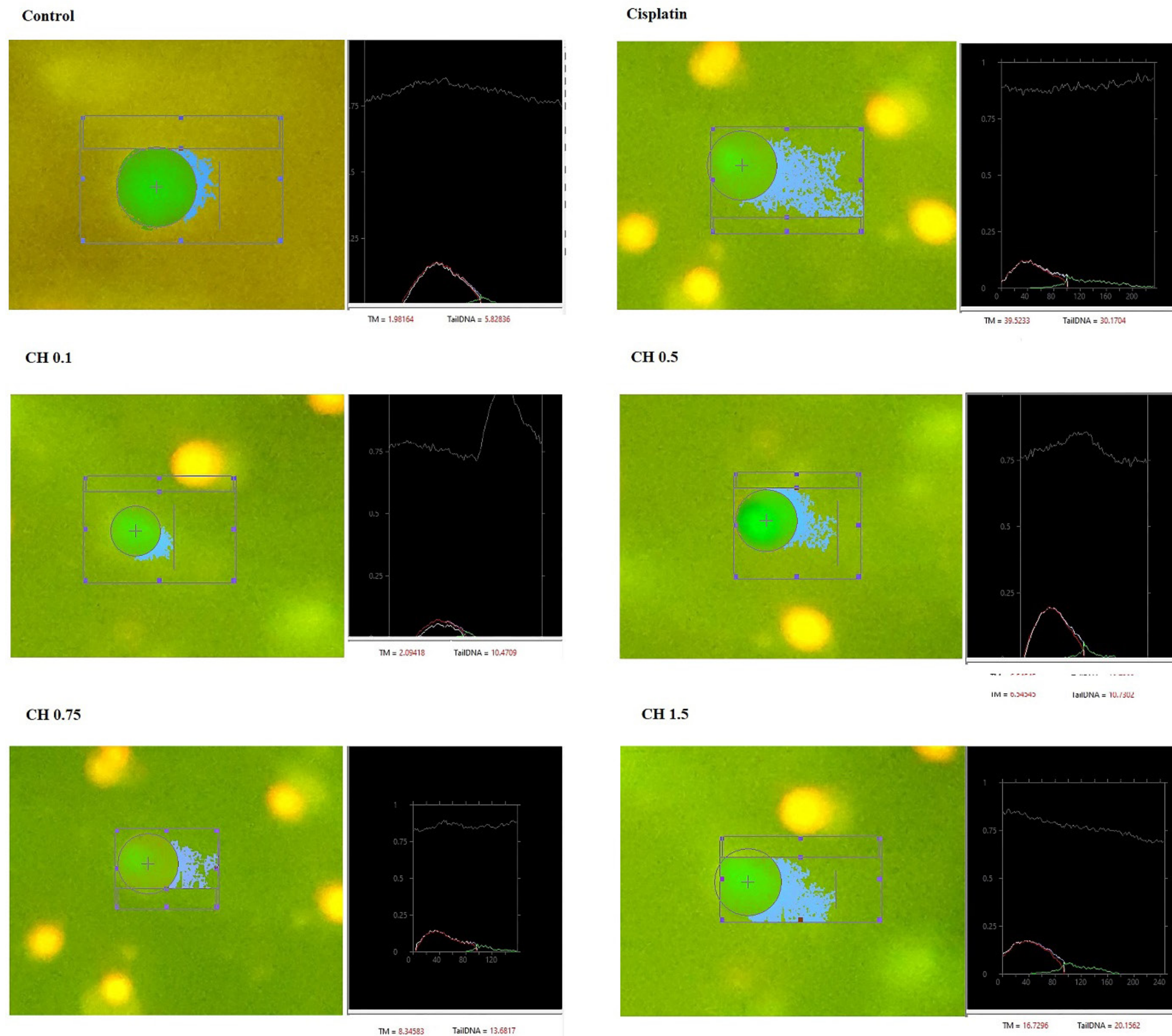

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Figure 1. Genotoxicity in comet assay in human lymphocytes: The increased DNA in the tail was observed in cisplatin and chlorpheniramine (1.5 mM) groups. CH: Chlorpheniramine.

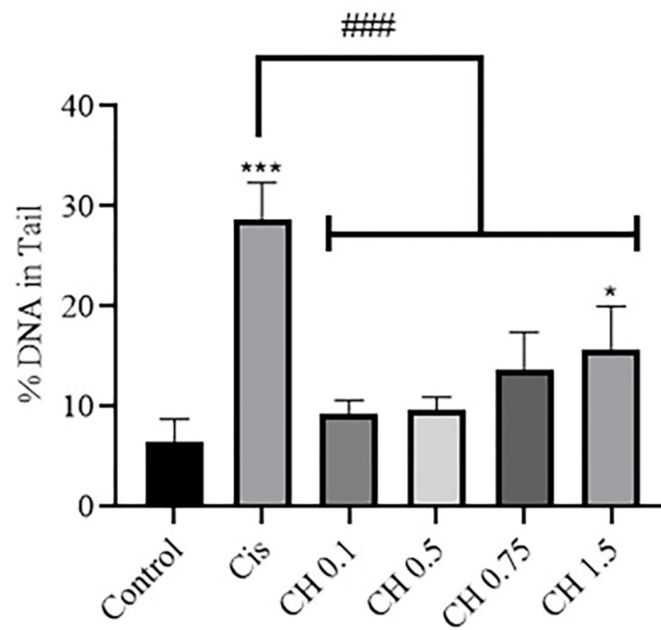

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Figure 2. The percentage of DNA in the tail in human lymphocytes after treatment with chlorpheniramine; Values are presented as Mean±SD; *Significantly different compared to the control group (P<0.05); ***Significantly different compared to the control group (P<0.001); ###Significantly different in comparison with the cisplatin group (P<0.001). CH: Chlorpheniramine (0.1, 0.5, 0.75, and 1.5 mM); Cis: Cisplatin (µg/mL).

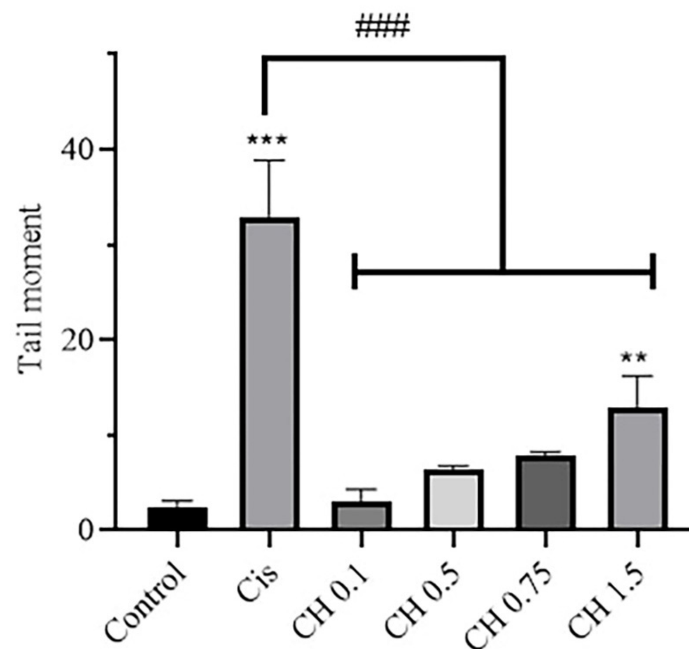

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Figure 3. The changes in the percentage of the tail moment in human lymphocytes after treatment with chlorpheniramine. Values are presented as Mean±SD; **Significantly different compared to the control group (P<0.01); ***Significantly different compared to the control group (P<0.001); ###Significantly different in comparison with the cisplatin group (P<0.001). CH: Chlorpheniramine (0.1, 0.5, 0.75, and 1.5 mM); Cis: Cisplatin (µg/mL).

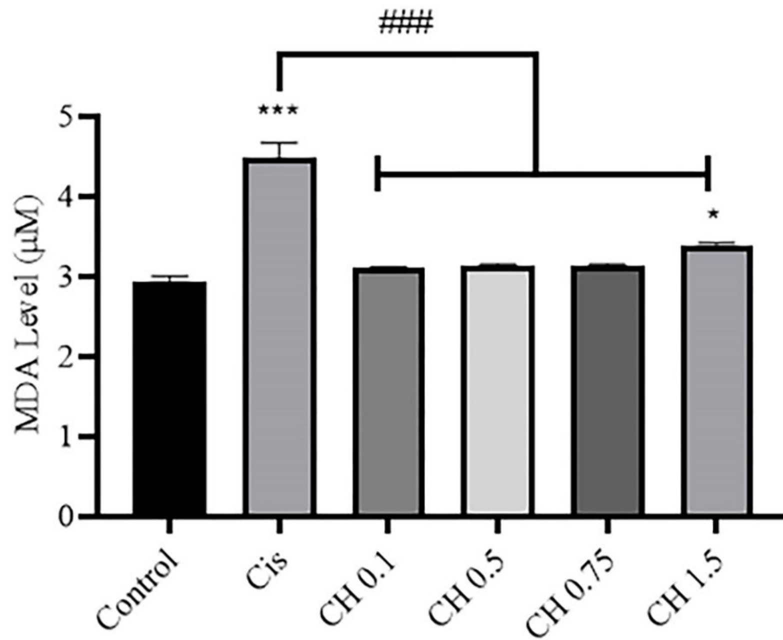

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Figure 4. Induction of lipid peroxidation in human lymphocytes after treatment with high concentrations of chlorpheniramine. MDA concentrations were evaluated as a by-product of lipid peroxidation; Values are presented as Mean±SD; *Significantly different compared to the control group (P<0.05); ***Significantly different compared to the control group (P<0.001); ###Significantly different in comparison with the cisplatin group (P<0.001). CH: Chlorpheniramine (0.1, 0.5, 0.75, and 1.5 mM); Cis: Cisplatin (µg/mL).

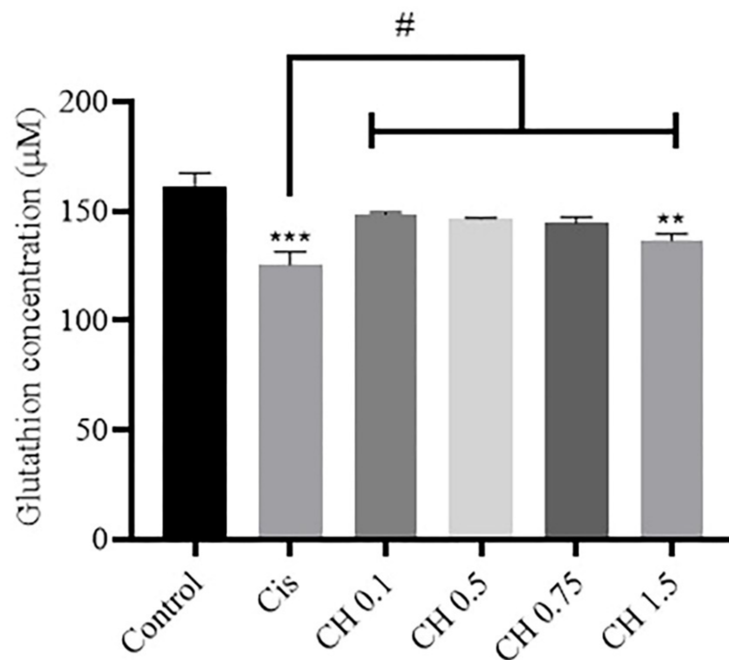

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Figure 5. Effect of high concentrations of chlorpheniramine on the levels of glutathione; The key role of oxidative stress in chlorpheniramine-induced genotoxicity. **Significantly different compared to the control group (P<0.01); ***Significantly different from the control group (P<0.001); ###Significantly different compared to the cisplatin group (P<0.001). CH: Chlorpheniramine (0.1, 0.5, 0.75, and 1.5 mM); Cis: Cisplatin (µg/mL).

ice bath, and simultaneously, n-butanol was added to all groups. In this stage, the samples were centrifuged for 10 minutes at 3500×g. Finally, the supernatants were evaluated with three replicates at 532 nm using a microplate reader. MDA level was expressed as μM.

Measurement of glutathione content

The assessment of glutathione content was conducted as it was claimed earlier [27]. In this assay, DTNB (5,5-dithiol-bis-(2-nitrobenzoic acid) was used as an indicator to determine glutathione concentration. As for the first stage, the protein content of each cell suspension was precipitated after the addition of TCA. Then, all samples were centrifuged for 5 minutes at 5000×g and 4°C. Subsequently, DTNB and PBS were added to the supernatant of each sample. At last, the final mixture was analyzed by a spectrophotometer at 412 nm. Total glutathione concentration was expressed as μM glutathione.

Statistical analysis

All statistical analyses were performed using GraphPad Prism® software, version 6. The results were expressed as Mean±SD. The assays were performed in at least triplicate. Comparison between groups was made using the one-way ANOVA, followed by the post hoc Tukey's test. A P<0.05 was considered statistically significant.

Results

Alkaline comet assay

Alkaline comet assay was conducted in this study as a sensitive method to measure the DNA damage caused by chlorpheniramine. Figure 1 demonstrates the comet formed in each group compared to the control group. The intensity of damaged DNA in the tail is revealed at the concentration of 1.5 mM and in the cisplatin group (Figure 1). The results of this assay are expressed as the DNA percentage in the tail (Figure 2) and tail moment (Figure 3). As it is indicated in both figures, there were significant increases in the DNA percentage in the tail (P<0.05) and tail moment (P<0.01) at the concentration of 1.5 mM in comparison with the control group. Also, the increases in the DNA percentage in tail and tail moment are statistically significant compared to the cisplatin group as the positive control (P<0.001).

Measurement of oxidative stress parameters

Measurement of lipid peroxidation

MDA levels were quantified in this study as it is considered to be the by-product of lipid peroxidation. As shown in Figure 4, a significant increase was observed in the levels of MDA at the concentration of 1.5mM compared to the control group (P<0.05) and to the cisplatin group as the positive control (P<0.001).

Measurement of glutathione content

In this study, the levels of glutathione were measured as another biomarker of oxidative stress. Figure 5 indicates that glutathione levels decreased significantly at the concentration of 1.5 mM in comparison with the control group (P<0.01) and the cisplatin group as the positive control (P<0.001).

Discussion

Chlorpheniramine is a classic and short-acting alkylamine antihistamine, which has a wide variety of indications [28]. In clinical practice, chlorpheniramine is extensively prescribed to reduce the incidents associated with infusion reactions [12, 29]. Similar to other drugs of this family, chlorpheniramine is of alleviates effects upon diverse allergic as well as non-allergic conditions. The extensive consumption of chlorpheniramine can be followed by several common side effects, including somnolence, and impairment of cognitive and motor function even at low doses of the drug [30]. However, it is assumed that chlorpheniramine might have the potential to induce less frequent adverse events, such as liver injury and genotoxicity [14-16, 31]. Oxidative stress is speculated to be one of the major mechanisms associated with these side effects [14].

The purpose of the current study was to determine the possible potential of chlorpheniramine in inducing genotoxicity in human peripheral lymphocytes and highlight the probable role of oxidative stress in this event as the principal mechanism.

In this experiment, we conducted an alkaline comet assay to detect damage to the genetic components of the cells. The alkaline comet assay is regarded as a simple and sensitive method with the ability to detect DNA strand breaks and DNA repair as well as DNA-DNA and DNA-protein crosslinks [32, 33].

Our findings demonstrated that chlorpheniramine could induce genotoxicity at high concentrations (1.5 mM, $P<0.05$) evidenced by significant increases in the DNA percentage in tail and tail moment. In the same context, a micronucleus assay was conducted on Chinese hamster lung V79 cell lines and it was observed that chlorpheniramine increased the percentage of micronucleated cells significantly at 500 µg/mL (1.81 mM) in the absence of S9 indicating that chlorpheniramine might have an intrinsic feature to interact with DNA [16]. Interestingly, the genotoxicity occurred at a high concentration similar to our results. Moreover, several studies have provided further evidence of the ability of chlorpheniramine to induce genotoxic effects in the chromosome aberration test (ABS) on Chinese hamster ovary cells (CHO) at the concentration of 500 µg/mL (1.81mM) in the presence of an exogenous metabolic enzyme (S9) [34, 35].

In another study, alkaline pulse-field gel electrophoresis was conducted on the rat hepatocytes, which were treated with relatively similar concentrations of chlorpheniramine (0.1, 0.25, 0.5, 0.75, and 1 mM) compared to our work. Unlike our findings, it was observed that in a dose-dependent way, chlorpheniramine significantly induced DNA strand breaks at 0.5, 0.75, and 1 mM. Also, based on the results of the trypan blue dye exclusion assay conducted in the same study, it was reported that chlorpheniramine might be cytotoxic in the concentrations of 0.5, 0.75, and 1 mM. Therefore, the DNA fragmentations, which were observed alongside the DNA strand breaks can be attributed to the activation of endonucleases in the apoptotic cells [15]. While in our study, the concentrations of 0.5 and 0.75 mM did not exhibit significant effects. We assume that the exposure time and different cell lines can be possible reasons that the results of our study are different because DNA strand breaks were evaluated 1 hour and 48 hours after treating rat hepatocytes, while we assessed the results after 24 hours in human lymphocytes. In addition, the results of the mutagenicity assay on Salmonella typhimurium showed that this drug does not have mutagenic properties [36, 37]. In accordance with these studies, chronic administration of chlorpheniramine for two years on male and female albino mice (B6C3F1) and rats (F344/N) did not provide significant evidence of carcinogenicity [38].

Based on the presence of two unfused rings and a dimethyl amino cationic group in the structure of chlorpheniramine, it is hypothesized that chlorpheniramine may exhibit DNA intercalating properties and this feature probably plays a principal role in the genotoxicity induced by chlorpheniramine [16, 39-42].

The major involvement of oxidative stress and ROS in the DNA damages induced by various DNA intercalators has been assessed in different studies [43-45]. As regards chlorpheniramine, outlining the key role of oxidative stress in the induction of genotoxicity was our main aim. Consistent with our study, chronic administration of chlorpheniramine for three weeks in male albino mice is correlated to significant increases in the levels of MDA and hepatic enzymes as well as significant depletion in the levels of glutathione and albumin. However, treating the mice with L-carnitine and chlorpheniramine resulted in lower levels of MDA and liver enzymes in addition to increases in glutathione and albumin levels. According to the results of this study, it can be presumed that chlorpheniramine might potentiate hepatotoxicity through oxidative stress [14]. Similarly, in our study, we observed that chlorpheniramine increased MDA and decreased glutathione levels at high concentrations (more than 1.5 mM).

Conclusion

In this study, we found no significant evidence that low concentrations of chlorpheniramine (0.1, 0.5, and 0.75 mM) could induce genotoxicity. On the other hand, at high concentrations (1.5 mM), chlorpheniramine significantly mediated the genotoxic event. In addition, we showed that oxidative stress was one of the major mechanisms involved in chlorpheniramine-induced genotoxicity.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the ethical committee of Guilan University of Medical Sciences, Rasht, Iran (Code: IR.GUMS.REC.1400.555).

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Authors' contributions

Conceptualization and Methodology: Ehsan Zamani and Mehdi Evazalipour. Experimentation: Saba Mahboubi, Ehsan Zamani and Mehdi Evazalipour. Data analysis: Ehsan Zamani. Writing the manuscript: Saba Mahboubi.

Conflict of interest

The authors declared no conflicts of interest.

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