Cytotoxic and Genotoxic Effects of Sulfonamide-Aldehyde Derivative in Allium cepa Root Tip Cells

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ABSTRACT

The continuous production and release into the environment of chemicals has revealed the need to determine their cytotoxicity and genotoxicity. Sulfonamide-aldehyde (SA) derivatives, whose biological activity properties vary in a wide spectrum, are frequently used in agriculture, medicine, pharmacy and many other fields. These compounds have an important cycle in the ecological system due to their use and diversity. In the present study, the potential cytotoxic and genotoxic effects of sulfonamide-aldehyde derivative were investigated using Allium test system with the concentrations of 6.25, 12.5, 25, 50, and 100 µM. In A. cepa cells, the increasing concentrations of SA caused cytotoxic effects by inducing nuclear lesions and inhibition of mitotic index. In addition, the increasing concentrations of SA caused genotoxic effects by inducing micronucleus and chromosome aberrations, which the most common ones are C-mitosis, sticky metaphase, and anaphase bridge. The results indicate that the concentration of 25 µM is EC50 in micronucleus, nuclear lesions, and chromosome aberrations; and 50 µM is EC50 in mitotic index (p < 0.05).

Keywords: Allium Test, Azo-Dyes, Chromosome Aberration, Micronucleus, Mitotic Index

Allium cepa Kök Ucu Hücrelerinde Sülfonamid-Aldehit Türevinin Sıtotosik ve Genotosik Etkileri

ÖZET

Kimyasalların sürekli üretimi ve çevreye salınmaları sitotositsitelerini ve genotositsitelerini belirleme ihtiyacı ortaya çıkarmıştır. Biyolojik aktivite özellikleri geniş bir spektrumda değişen sülfonamid türevleri, tarm, tp, eczacılık ve diğer birçok alanda sıkıla kullanılmaktadır. Bu bileşikler, kullanımları ve çeşitlilikleri nedeniyle ekolojik sisteme önemli bir döngüye sahiptir. Bu çalışmada, sülfonamid-aldehid (SA) türevinin potansiyel sitotosik ve genotosik etkileri 6.25, 12.5, 25, 50 ve 100 µM konsantrasyonlarında Allium test sistemini kullanılarak araştırılmıştır. A. cepa hücrelerinde, artan SA konsantrasyonları mitotik indeksi inhibe ederek ve nükleer lezyonları indükleyerek sitotosik etkilere neden oldu. Ek olarak, artan SA konsantrasyonları, en yaygın olanları C-mitoz, yapışkan metafaz ve ana faz köprüsü olan kromozom anomalileri ve mikronükleus anomalileri indükleyerek genotosik etkilere neden oldu. Sonuçları gösteriyor ki mikronükleus, nükleer lezyonlar ve kromozom anomalilerinde 25 µM’lik konsantrasyon ve mitotik indekste ise 50 µM’lık konsantrasyon EC50’dir (p <0.05).

Anahtar Kelimeler: Allium Testi, Azo-Boyalar, Kromozom Kusuru, Mikronükleus, Mitotik İndeks

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I. INTRODUCTION

Azo compounds (azo dyes) are organic compounds containing the group -N=N-. These compounds are used in textile, pharmaceutical, paper, plastic, paint, and cosmetics industries. In addition, they are used in chemical analyzes (as indicators), in printing (as ink), in biological research (in cell staining), and in foods (as colorants) [1,2].

The coupling reaction performed to form an azo group is called azo coupling. This reaction takes place between a diazonium salt composed of an aromatic primary amine and an aromatic coupling component carrying a substituent such as -OH, -NH₂, -NH (R). Aromatic diazonium salts are effective electrophiles against aromatic compounds containing strong electron donating groups. Due to its electrophilic properties, diazonium salt can react with nucleophile compounds, namely aromatic primary, secondary and tertiary amines, naphthol and phenol compounds. In short, the synthesis of azo dyes takes place in two stages with diazotization and coupling reactions [3].

The sulfa drugs, known as sulfonamides, are antibacterial drugs that have been used alone for many years in many indications due to their wide spectrum. Sulfonamides, which are a specific family of organic sulfur compounds and represented by the general formula R-SO₂NH₂, are widely used in medicine, agriculture, veterinary medicine, pharmacology, biotechnology, and industry. The study by Gerhard Domagk using a dye called prontosil (Figure 1) on the mice in 1932 is the first to make sulfonamide compounds popular. Domagk examined the in vivo activity of this red colored compound and used it for therapeutic purposes in blood poisoning [4,5]. The sulfasalazine (azulfidine), sulfamethoxazole (gantanol), sulfapiridine, sulfacetamide, and sulfadiazone, which are derivatives of sulfonamide and have a commercial market share, are important drug examples [6]. In addition, sulfonamide derivative are important compounds used as plant growth regulator [7].

The Allium test was first conducted by Levan (1938) to determine the effect of colchicine. Then, certain modifications were made to this basic test system to improve its use [8-11]. Fundamentally, all the modifications proposed so far, are related to cell division and chromosomal defects. The categories that are likely to be analyzed with the Allium test system are listed. Mitotic index (MI) refers to the total number of dividing cells in the cell cycle and is used to determine the cytotoxic effect. Micronucleus (MN) originates from chromosome breaks or chromosome losses and is very similar to the nucleus.
Nuclear abnormalities (NA) express the morphological changes in the interphase nucleus. The abnormal forms of nucleus are effective indicators of cytotoxicity. Chromosome aberrations (CA) refers to the alterations in total chromosome number or chromosome structure and is characterized by many parameters, which are bridge, adherence, break, loss, fragment, multipolar, C-metaphase, vagrant etc [12]. The aim of this work was to investigate the cytotoxicity and genotoxicity of sulfonamide-aldehyde derivative (SA) (Figure 1) in the root tip cells of *Allium cepa* L.

II. MATERIALS AND METHODS

A. MATERIALS

The target molecule (SA) containing sulfonamide and aldehyde groups was previously synthesized in two steps by our groups [13]. In the synthesis of the target compound, firstly, the enamino molecule was synthesized as the starting compound. In this synthesis, 4-methylacetophenone (1 mmol) and DMF-DMA (1.5 mmol) reagents were used. This reaction was carried out by refluxing in xylene solvent for 24 hours. Then, the aromatic amine compound (sulfanilamide) was converted into diazonium salt in acidic medium at 0 °C using sodium nitrite reagent. Yellow colored diazo compound (SA) was obtained from the reaction of diazoum salt and enamino compound at 0 °C. In this reaction carried out in ethanol solvent, the compound SA precipitated in the reaction medium within 5 minutes. Compound SA, whose cytotoxic and genotoxic effects were studied, was purified by crystallization from n-butanol solvent [13]. SA is a tautomer mixture (keto-hydrazo and enol-azo) and its structural formula is given in Figure 1. IUPAC name of SA is 4-(2-(1,3-dioxo-1-(p-tolyl) propan-2-ylidene)hydrazinyl)benzenesulfonamide. The molecular formula is C_{16}H_{15}N_{3}O_{4}S and the melting point is 188-190°C.

The plant materials (*A. cepa, 2n = 16*) were obtained from the local market (Yozgat, Turkey). In addition, all chemicals were purchased from Merck and Sigma-Aldrich.

B. ALLIUM TEST

*A. cepa* bulbs were germinated in test tubes at room temperature until reach a length of about 1 cm. Then, the roots were treated with different concentrations of SA (6.25, 12.5, 25, 50, and 100 µM) for a period of 48 h. No concentration was applied to the roots forming the control group. Then, the roots were fixed in fixative solution (ethanol:acetic acid, 3:1 v/v) for 24 h. Then the roots were transferred into 70% alcohol and stored at 4 ºC until analysis. At analysis time, the roots were hydrolyzed in 1N HCl at 60 ºC for 8 min and washed with distilled water. Root tips were stained in aceto-orcein for 2h and squashed in 45% acetic acid [9,10].

C. OBSERVATIONS AND CALCULATIONS

In microscopic analysis, six slides for each treatment were observed under the light microscope (Olympus BX53, Japan) at 400× magnification and photographed with a digital camera (Olympus DP72, Japan). About 3000 cells were analyzed for each concentration in the evaluation of MI, MN, and nuclear lesions (NL). The MI, MN, and NL were calculated as follow:

\[
\text{MI} \text{ (%) } = \left( \frac{\text{Total number of cells in mitosis}}{\text{Total number of cells}} \right) \times 100
\]

\[
\text{MN} \text{ (%) } = \left( \frac{\text{Total number of micronuclei}}{\text{Total number of cells}} \right) \times 100
\]

\[
\text{NL} \text{ (%) } = \left( \frac{\text{Total number of nuclear lesions}}{\text{Total number of cells}} \right) \times 100
\]

At least 3500 cells were analyzed for each concentration in the evaluation of CA. The CA was calculated as follow:
CA (%) = (Total number of chromosomal defects / Total number of cells) × 100 [15].

D. STATISTICAL ANALYSIS

The data were calculated by one-way analysis of variance (ANOVA) and differences among groups were determined by the Tukey test (p < 0.05) in MS-Excel software. In addition, all parameters and variables were compared by Pearson correlations (PC), which are weak correlation (PC ≤ 0.25), average correlation (0.25 < PC ≤ 0.50), good correlation (0.50 < PC ≤ 0.75), and high correlation (0.75 < PC).

III. RESULTS AND DISCUSSION

Table 1 shows the results of MI, MN, NL, and CA in A. cepa cells. After exposure to SA, the MI rates are 3.04 ± 0.26 in 50 µM and 2.04 ± 0.20 in 100 µM, and they are considerably lower than the control group (8.07 ± 0.47). Increasing SA concentrations have caused decreasing in MI rates (p < 0.05).

Table 1. The results of mitotic index, micronucleus, nuclear lesions, and chromosome aberrations in Allium cepa cells after exposure to SA concentrations (µM).

| Concentrations | MI     | MN     | NL     | CA     |
|----------------|--------|--------|--------|--------|
| Control        | 8.07 ± 0.47 | 0.60 ± 0.06 | 1.32 ± 0.11 | 0.70 ± 0.06 |
| 6.25           | 5.56 ± 0.35 | 1.03 ± 0.11 | 2.26 ± 0.16 | 1.26 ± 0.06 |
| 12.5           | 5.06 ± 0.34 | 1.59 ± 0.14 | 2.23 ± 0.18 | 1.72 ± 0.13 |
| 25             | 5.10 ± 0.32 | 2.61 ± 0.20* | 4.08 ± 0.22* | 2.23 ± 0.23* |
| 50             | 3.04 ± 0.26* | 2.56 ± 0.18* | 5.23 ± 0.31* | 2.16 ± 0.21* |
| 100            | 2.04 ± 0.20* | 3.62 ± 0.23* | 5.17 ± 0.26* | 3.26 ± 0.31* |

* indicate significant differences between control and concentrations. Significance at p < 0.05.

The treatment with 25, 50, and 100 µM concentrations of SA significantly (p < 0.05) increased the percentage of MN and NL in mitotic cells compared to the control (Table 1). Figure 2 presents the micronuclei and lesions in the meristematic cells of A. cepa.

Figure 2. The meristematic cells of Allium cepa after exposure to SA. (a) nuclear lesions (arrows); (b) micronucleus (arrow). Magnification 400×

Figure 3 shows the normal stages of cell division and CA in meristematic cells of A. cepa after exposure to SA. CA are detected as anaphase bridge, sticky metaphase, sticky anaphase, C-mitosis, chromosome fragments, multipolar anaphase, diagonal anaphase, vagrant chromosome, ring chromosome, lobulated nucleus, binucleate cell, and polyploid cell. The types and numbers of CA are given in Table 2. The
most common chromosomal defects are C-mitosis, sticky metaphase, and anaphase bridge, respectively. The CA rates are $2.23 \pm 0.23$ in 25 µM, $2.16 \pm 0.21$ in 50 µM, and $3.26 \pm 0.31$ in 100 µM; and they are considerably higher than the control ($0.70 \pm 0.06$) and other groups. Increasing SA concentrations have caused increasing in CA rates ($p < 0.05$) (Table 1).
Table 3 presents the Pearson correlations for all parameters and variables. The negative high correlations are showed between MI and other three parameters, which are MN (r = -0.900), NL (r = -0.904), and CA (r = -0.921) (p < 0.05). The positive high correlations are showed between MN and four parameters and variables, which are NL (r = 0.934), CA (r = -0.986), sticky chromosome (r = 0.948), and C-mitosis (r = 0.952) (p < 0.05). In addition, NL are showed the high correlation with CA (r = 0.887, p > 0.05).

Table 2. The types and numbers of chromosome aberrations observed in meristematic cells of Allium cepa after exposure to SA.

| Chromosome aberrations | Control | 6.25 | 12.5 | 25  | 50  | 100 |
|------------------------|---------|------|------|-----|-----|-----|
| Anaphase bridge        | 2       | 6    | 14   | 18  | 12  | 12  |
| Sticky metaphase       | 4       | 6    | 5    | 16  | 19  | 38  |
| Sticky anaphase        | 3       | 6    | 5    | 25  | 24  | 27  |
| C-mitosis              | 5       | -    | 1    | 4   | 1   | 5   |
| Chromosome fragment    | 1       | 2    | 8    | 7   | 27  | 5   |
| Multipolar anaphase    | 4       | 2    | 4    | 1   | 5   | 1   |
| Diagonal anaphase      | -       | 1    | -    | 3   | 3   | 1   |
| Vagrant chromosome     | -       | 4    | 2    | 4   | 6   | 1   |
| Ring chromosome        | 1       | 2    | -    | 1   | 2   | 1   |
| Lobulated nucleus      | 2       | -    | 2    | 1   | 1   | 1   |
| Binucleate cell        | 2       | 3    | 2    | 2   | 1   | 1   |
| Polyploid cell         | 2       | 3    | 2    | 2   | 1   | 2   |
| Total number of abnormal cells | 21 | 39 | 56 | 75 | 72 | 104 |

Table 3. The Pearson correlations for parameters and variables.
Correlation is significant at the 0.05 level (bold values). Abbreviations: anaphase bridge (AB), sticky chromosome (SC), C-mitosis (CM), chromosome fragment (CF), multipolar anaphase (MA), diagonal anaphase (DA), vagrant chromosome (VC), ring chromosome (RC), lobulated nucleus (LN), binucleate cell (BC), and polyploid cell (PC).

The MI, is a rate of mitotic cells in the cell cycle, is an indicator of cell proliferation. Low MI rates compared to negative control may express the effect of the chemical as a cytotoxic potential on the growth and development of the organism exposed to the chemical. In the present study, the MI rates are considerably lower than the control group and this condition may induce the CA. In Table 3, the high negative correlation between MI and CA indicates this. That is, the lower the MI, the higher the CA.

CA is an effective parameter to identify possible genotoxic potentials of chemicals and reveal important data in this regard [16]. The most observed CA at all concentrations of SA are C-mitosis, chromosome stickiness, and anaphase bridge. These defects may be due to the effect of SA on spindle formation and cause disturbed phase on cell division. Leme and Marin-Morales (2009) reported that C-mitosis and vagrant chromosomes are possible sources of aneuploidy. They also recorded that chromosome bridges cause chromosome breaks and consequently a clastogenic effect [12]. In addition, sticky chromosomes can cause anaphase bridges and as a result remain connected with these bridges and has a high genotoxic potential that extends to cell death [15,17]. Table 3 presents a positive correlation between chromosome stickiness and anaphase bridge (r = 0.456).

The polyploid cells may increase in the presence of C-mitosis and vagrant chromosomes that affect the function of the spindle apparatus [18]. In the present study, observed polyploid cells are low and the correlations are positive between polyploid cells and vagrant chromosome (r = 0.472) and weak negative between polyploid cells and C-mitosis (r = -0.230) (Table 3).

The MN assay is one of the most reliable tests used to investigate to the genotoxicity. MN rates are higher than control group in interphase cells. In addition, MN assay is an important parameter in determining the aneugenic, genotoxic, and clastogenic effects in A. cepa, which has symmetric karyotype [19]. The symmetric karyotype is characterized by high rates of median and submedian chromosomes [20]. MN rates are higher than control group in interphase cells. The fragment or vagrant chromosomes induce micronucleus formation [16]. Table 3 presents the positive correlations between MN and vagrant chromosome (r = 0.713) and between MN and chromosome fragment (r = 0.358).

In literature, there are records on the cytotoxicity and genotoxicity of sulfonamides. Özkan and Liman reported that the penoxsulam, is a sulfonamide herbicide, showed a cytotoxic effect by reducing MI, a genotoxic effect because it increased CA, which are stickiness, anaphase bridge, disturbed anaphase-telophase, chromosomal laggards, and polyploidy [7]. Badr (1982) analysed the cytogenetic effects of 3 sulphonamides, which were sulphadiazine, sulphadimidine, and sulphanaphenazole and showed the mitotic and chromosomal abnormalities [21]. In addition, Leme and Marin-Morales (2009) presented the detailed review regarding A. cepa test in environmental monitoring and recorded the cytotoxicity and genotoxicity of the metals, pesticides, aromatic hydrocarbons, textile industry dyes, products used to disinfect drinking water, complex mixtures, and other agents [12].

The results of this study are similar to the above studies in terms of cytotoxic effect by reducing MI and genotoxic effect by increasing CA. Especially, the chromosomal defects as bridges, stickiness, C-mitosis, lagging, and vagrant are important similarities. In addition, MN and NL data support the genotoxicity status.

The cytotoxic, genotoxic, anticytotoxic, and antigenotoxic potentials of the sulfonamide chalcone were determined using the ames test and the mouse bone marrow micronucleus test [22]. Şen at al. (2017) aimed to assess the genotoxic potentials of two new synthesized sulfonamide derivatives. According to the obtained results, the test substances are cytotoxic at high concentrations and long-term exposure but they are not genotoxic in human peripheral lymphocytes [23]. Samadai et al. (2020) reported the cytotoxic activity of chiral sulfonamides based on the 2-azabicycloalkane skeleton [24].
As a result, in the current study, it has been determined that a decrease in the dose amount has negative effects on cell cycle proliferation and causes many and different types of CA. The SA induced MN, NL, and aberrations as bridges, stickiness, C-mitosis, chromosome fragment, multipolar anaphase, diagonal anaphase, vagrant chromosome, ring chromosome, lobulated nucleus, binucleate cell, and polyploid cell in Allium root tips. We suggested that the test substances were genotoxic and cytotoxic in high concentrations.

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