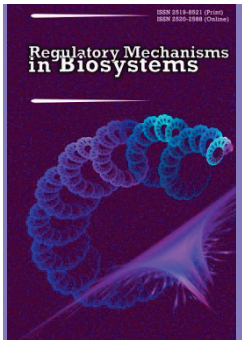

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Cyto/genotoxicity of potassium metabisulfite and antimutagenic effect of piperine

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Potassium metabisulfite is a white crystalline powder used in wine production and food preservation. Piperine is an alkaloid of the piperidine series and it is the main ingredient of black pepper. The aim of the present study was to evaluate the cyto/genotoxic effects of potassium metabisulfite and antimutagenic effect of piperine using the *Allium* test. To test the cyto/genotoxicity of potassium metabisulfite, three concentrations were used: 0.5, 1.0 and 2.0 g/L. To evaluate the antimutagenic effect of piperine, the following concentrations were applied: 3.3, 6.6 and 13.3 µL/mL. Our results demonstrated that all three tested concentrations of potassium metabisulfite significantly reduced root growth. Mitotic index values significantly decreased after the treatment with all three concentrations of potassium metabisulfite. An increased frequency of apoptotic cells was observed for the two highest concentrations of potassium metabisulfite, while a statistically different frequency of necrosis was detected for all three potassium metabisulfite concentrations in comparison with control. The total number of cytotoxic markers differed between all three potassium metabisulfite concentrations and control. A significantly higher frequency of sticky chromosomes for the two highest potassium metabisulfite concentrations, as well as an increased number of chromosomal bridges for median potassium metabisulfite concentration as compared to control group was determined. A significantly higher number of binuclear cells was detected for the highest potassium metabisulfite concentration. A significant difference between the two highest potassium metabisulfite concentrations and control was established for the total number of aberrant cells. As for the results for piperine, a reduction in the roots' growth was observed between the two highest piperine concentrations and negative control. The mitotic index decreased after treatment with all three piperine concentrations in comparison with negative and positive control. A significant difference in the frequency of apoptosis between median piperine concentration and positive control was detected. An increased frequency of necrosis between the two highest piperine concentrations and both controls was determined. The total number of cytotoxicity markers increased between the highest piperine concentration and both controls. A significantly higher frequency of chromosomal bridges between the two highest piperine concentrations and negative control was observed. The total number of aberrant cells differed between two highest piperine concentrations and negative control. This study demonstrated that potassium metabisulfite manifests antiproliferative, cytotoxic and genotoxic effects. Similarly, antiproliferative properties of piperine have been observed, while its antimutagenic potential has not been demonstrated. Further studies are needed to strengthen these observations.

Keywords: potassium metabisulfite; piperine; *Allium* test; toxicity; antiproliferative potential.

Introduction

As the human population increases day by day, the amount of food needed to feed our species also increases. The most logical way to increase the amount of food available for human consumption, without actually producing more food, is to reduce the amount of unconsumed food that expires or spoils to the point where it is no longer safe for human consumption (Rigby, 2019). Preservatives are substances that extend the shelf life of foods and protect them from spoilage due to microbial, chemical or physical actions (Amit et al., 2017). In general, preservatives should be active at low concentrations, protect products from a wide range of microorganisms, should not interact with other ingredients of the product and aggravate its quality, and should not be toxic to humans and environment (Liao et al., 2013). However, some research has shown that various food additives, especially chemical preservatives and antimicrobial agents are genotoxic in different test systems (Pandey et al., 2014). Genotoxic substances, through their mutagenic and carcinogenic effects, cause changes in the genome that can have negative consequences for the normal functioning and reproduction of the organism (Ren et al., 2017). Bioactive plant extracts are an integral part of folk medicine and often exhibit multiple biological activities including antioxidant and antimutagenic activities. This led researchers to a systematic evaluation of various biological properties of medicinal plants (Khan & Ahmad, 2020).

Potassium metabisulphite is commonly used as a preservative to maintain the stability of wines over time (Varo et al., 2022). Potassium metabisulphite is an inorganic salt that dissociates in an acid medium, such as wine, to generate sulfur dioxide. This compound is used as an antibacterial, antilevadurian and antioxidant (Garaguso & Nardini, 2015), as well as to control enzymatic darkening reactions in red wines (García-Guzmán et al., 2015). In this regard, the appropriate use of this compound results in less oxidized wines, having better color and aroma, and lower volatile acidity. However, it is well known that potassium metabisulphite changes the color of the wines, since it can interact with the compounds present in the medium, such as, anthocyanin monomers, causing an alteration from red to colorless, or it can cause polymerization of these compounds creating those resistant to SO_2 decolorization (Varo et al., 2022).

Piperine is an amide alkaloid of the piperidine series and it is the main secondary metabolite of black pepper (*Piper nigrum* L.) and long pepper (*Piper longum* L.) fruit (Lee et al., 2021). It has been demonstrated that *Piper nigrum* fruit contain 3,000–6,650 mg/100 g of piperine, while its root contain 790 mg/100 g of piperine (Lee et al., 2021). Piperine and other alkaloids from the species *Piper nigrum* possess numerous pharmacological and antimutagenic properties. Piperine has fundamental effects on p-glycoprotein and many enzyme systems, leading to biotransformational effects including chemoprevention, detoxification, and improving the absorption and bioavailability of herbal and conventional drugs. Based

on modern studies on animal cells, piperine has been found to have immunomodulating, antioxidant, anticancer, anti-inflammatory, anti-apoptotic and bio-availability enhancing properties (Meghwal & Goswami, 2013; Siddiqui et al., 2023).

The *Allium* test is a highly sensitive toxicological test that uses plant cells as test models. Using the *Allium* test, it is possible to establish the genotoxic or mutagenic effect of chemical compounds on the genome of higher plants. *Allium cepa* L. apical meristem cells are sensitive to the toxicity of substances, so macroscopic and microscopic parameters can be observed as indicators of cyto/genotoxic potential (Firbas & Amon, 2014).

Therefore, the aim of this study was to evaluate the cyto/genotoxic effects of potassium metabisulphite, as well as antimutagenic potential of piperine using *Allium* test.

Materials and methods

In the Soxhlet extraction apparatus, 30–40 g of ground black pepper was added. Ground black pepper was wrapped in filter paper, then ethanol was sprinkled over the pepper so that the solvent is transferred to a flask and heated in a water bath for two hours. The collected extract was concentrated on a rotavapor at a temperature of 60 ˚C. The residue after concentration was treated with 10–20 mL of 10% potassium hydroxide in ethanol, and kept in an ice bath. After ten minutes, the alcoholic solution was separated from the precipitate by filtration. The filtrate was allowed to cool in order to form the piperine crystals. The resulting crystals were washed with 2 mL of cold water. Piperine was then purified by recrystallization from an acetone-methanol solution (3:2), whereby opaque yellow crystals of piperine were formed.

For the detection of toxicological effects of tested substances, *Allium cepa* L. bulbs were used. The part of the bulb from which the layer of dried roots is removed was immersed in test tubes filled with fresh tap water for 48 hours, so that new roots would grow. The water was renewed every 24 hours. After 48 hours germination root length was measured. Afterward, the bulbs were treated with tested concentrations of potassium metabisulphite (0.5, 1.0 and 2.0 g/L) and piperine (3.3, 6.6 and 13.3 µL/mL) for 48 hours at room temperature. For each concentration of both test substances, as well as for the controls, series of four bulbs were used (quadruplicate). At the end of exposure period the root lengths of the bulbs were measured. Roots of bulbs whose length was greater than 1.5 cm were cut off and transferred to marked cuvettes. Then, 10 mL of freshly prepared fixative was added to each cuvette with the aim of stopping cell division. The cuvettes with the fixative and roots were left in the refrigerator for 48 hours at +4 ˚C. The fixative was prepared using 96% ethanol and glacial acetic acid (3:1 v/v).

After the end of the fixation period, the fixative was removed from the cuvette and roots were hydrolyzed in 3 mL of 0.1 M hydrochloric acid at 55 ˚C for 5 minutes. After that, roots were washed with distilled water and with the help of tweezers, they were transferred to the glass slides. The apical meristems of the roots were cut, placed in one drop of 2% acetoorcein, and then squashed. For each tested concentration of the potassium metabisulpfite and piperine, as well as for the control groups (positive and negative), four slides were prepared (one slide per bulb). Microscope slides were coded and analyzed. In this sense, 500 cells were analyzed per slide (in total 2000 cells per each test concentration and control groups). More precisely, the number of cells in mitotic division (mitotic index) was recorded. Mitotic index (MI) represents a measure of cell proliferation, and was calculated as the ratio between the cells in mitosis and the total number of cells analyzed. Furthermore, the frequency of cells with chromosomal abnormalities (genotoxic effects), as well as the frequency of cells with the cytotoxic markers (apoptosis and necrosis) was recorded.

Statistical data processing was performed by use of IBM SPSS Statistics for Windows, version 37 (Armonk, NY, USA). Mean values (x) and standard deviations (SD) were calculated for all analyzed parameters. In order to test significant differences between the experimental groups (potassium metabisulpfite and piperine test concentrations) and control groups, one-way ANOVA with post-hoc multiple comparison test (Tukey's test) was carried out. P values less than 0.05 were considered statistically significant.

Results

The results for the bulbs' root length after the treatment with potassium metabisulfite are presented in Table 1. Before the treatment, there were no significant differences in root length between any of the bulb groups. However, after the treatment all three tested concentrations of potassium metabisulfite significantly inhibited root growth when compared to the control group ($P < 0.05$). The greatest impact on root length inhibition was observed at the highest tested concentration of potassium metabisulfite. It is improtant to underline that there were no significant differences in root growth between tested concentrations of potassium metabisulfite.

Table 1
Bulb root length after treatment with different concentrations of potassium metabisulfite ($x \pm SD$, $n = 4$)

Concentration of potassium metabisulfite	Root length in the first 48 hours, mm	Root length after 48 hours, mm
Negative control (H_2O)	16.86 ± 7.06^{a}	35.21 ± 15.25^{a}
0.5 g/L	18.05 ± 3.35^{a}	17.26 ± 4.48^{bcd}
1.0 g/L	18.66 ± 3.65^{a}	17.70 ± 5.98^{cd}
2.0 g/L	16.41 ± 2.49^{a}	14.22 ± 1.18^{d}

Note: different lowercase letters indicate significant differences between the subgroups within one column ($P < 0.05$) according to the one-way ANOVA with Tukey's multiple comparison test.

The cytotoxic effects of potassium metabisulfite are presented in Table 2. It was found that the mitotic index (MI) significantly decreased after the treatment with all three tested concentrations of potassium metabisulfite as compared to the control group ($P < 0.05$). It is noticeable that the MI values were decreasing with an increase in the test substance. A significantly higher number of cells in apoptosis was observed between the two highest concentrations of potassium metabisulfite and the negative control ($P < 0.05$). Significantly increased apoptosis was observed between 0.5 and 1.0 g/L, as well as between 0.5 and 2.0 g/L of potassium metabisulfite. Statistically different frequency of necrosis was observed between all three concentrations of potassium metabisulfite and negative control ($P < 0.05$). However, no significant differences for the number of necrotic cells between studied concentrations of potassium metabisulfite was detected. Finally, the total number of cytotoxic markers was significantly different between all three tested concentrations of potassium metabisulfite and negative control ($P < 0.05$). The total number of cytotoxic markers increased with an increase in the concentration of the test substance.

Table 2
Cytotoxic effects in *Allium cepa* L. apical meristem cells treated with different concentrations of potassium metabisulfite ($x \pm SD$, $n = 4$)

Concentration of potassium metabisulfite	Mitotic index, %	Apoptotic cells	Necrotic cells	Total number of cytotoxic markers
Negative control (H_2O)	11.15 ± 2.58^{a}	0.25 ± 0.43^{a}	n.d^{a}	0.12 ± 0.33^{a}
0.5 g/L	2.70 ± 2.95^{bc}	0.75 ± 0.82^{a}	12.83 ± 4.60^{bcd}	8.00 ± 7.04^{bcd}
1.0 g/L	2.00 ± 1.58^{cd}	2.25 ± 0.43^{bac}	13.66 ± 5.09^{cd}	9.10 ± 7.30^{cd}
2.0 g/L	1.35 ± 1.78^{db}	2.00 ± 0.53^{ca}	19.00 ± 7.29^{d}	11.06 ± 9.79^{d}

Note: n.d. – not detected; see Table 1.

The results of genotoxic effects of potassium metabisulfite are summarized in Table 3. Regarding chromosomal aberrations, a significantly increased number of sticky chromosomes at the two highest concentrations of potassium metabisulfite, as well as an increased frequency of chromosomal bridges at median concentration of potassium metabisulfite in comparison with the negative control was observed ($P < 0.05$). No significant differences for the number of cells with sticky chromosomes, as well as for the number of cells with chromosomal bridges between studied concentrations of potassium metabisulfite, were demonstrated. A significantly higher number of binuclear cells was detected at the highest potassium metabisulfite concentration when compared to the control ($P < 0.05$). Similarly, a significantly increased frequency of binuclear cells between the lowest and the highest concentration of potassium metabisulfite was detected. As for the total number of observed aberrant cells, a statistically

significant difference between the two highest potassium metabisulfite concentrations and negative control was determined ($P < 0.05$). Also, the total number of observed aberrant cells differed significantly between 0.5 and 1.0 g/L, as well as between 0.5 and 2.0 g/L of potassium metabisulfite.

The results for the length of *A. cepa* roots treated with different concentrations of piperine are shown in Table 4. Before the treatment, no significant differences in root length between any of the bulb groups were detected. The weakest growth of roots after the treatment with piperine was observed at the highest tested piperine concentration, while the strongest root growth was detected at the lowest test concentration of piperine. Statistically significant difference in the root growth reduction was detected between the two highest piperine concentrations and negative control ($P < 0.05$). However, there were no observed significant differences in root growth among tested concentrations of piperine.

Table 3
Genotoxic effects in *Allium cepa* L. apical meristem cells treated with different concentrations of potassium metabisulfite ($x \pm SD$, $n = 4$)

Concentration of potassium metabisulfite	Sticky chromosomes	Chromosomal bridges	Vagrants	Chromosomal laggards	Breaks	Micronucleus	Nuclear bud	Binuclear cells	Total number of aberrant cells
Negative control (H_2O)	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	0.250 ± 0.433^{a}	0.031 ± 0.173^{a}
0.5 g/L	0.250 ± 0.433^{abc}	0.250 ± 0.433^{aba}	n.d.^{a}	n.d.^{a}	n.d.^{a}	0.250 ± 0.433^{a}	n.d.^{a}	0.500 ± 0.500^{aa}	0.156 ± 0.363^{a}
1.0 g/L	1.000 ± 0.707^{bc}	0.750 ± 0.433^{ba}	n.d.^{a}	0.500 ± 0.500^{a}	n.d.^{a}	n.d.^{a}	0.250 ± 0.433^{a}	1.000 ± 0.707^{ab}	0.437 ± 0.609^{bac}
2.0 g/L	1.000 ± 0.707^{c}	0.500 ± 0.500^{a}	0.500 ± 0.500^{a}	n.d.^{a}	0.250 ± 0.433^{a}	0.250 ± 0.433^{a}	0.250 ± 0.433^{a}	1.750 ± 0.433^{ba}	0.562 ± 0.704^{ca}

Note: see Table 2.

Table 4
Bulb root length after treatment
with different concentrations of piperine ($x \pm SD$, $n = 4$)

Concentration of piperine	Root length in the first 48 hours, mm	Root length after 48 hours, mm
Negative control (H_2O)	16.86 ± 7.06^{a}	35.21 ± 15.25^{a}
Positive control (EtOH) (13.3 µL/mL)	18.28 ± 6.77^{a}	30.30 ± 9.31^{abca}
13.3 µL/mL	15.31 ± 1.92^{a}	16.60 ± 1.84^{bca}
6.6 µL/mL	16.19 ± 1.31^{a}	19.74 ± 2.53^{ca}
3.3 µL/mL	18.44 ± 3.62^{a}	24.90 ± 5.04^{a}

Note: see Table 1.

The cytotoxic effects of tested concentrations of piperine are presented in Table 5. The mitotic index (MI) values significantly decreased after the treatment with all three tested concentrations of piperine when compared to both, negative and positive control ($P < 0.05$). The highest number of cells in mitosis was observed at the lowest piperine concentration, whereby MI decreased with an increase in the tested concentration. No significant differences in the number of apoptotic cells between any of the piperine concentrations and negative control were observed. On the other hand, significant difference for the frequency of apoptosis between median piperine concentration and positive control was demonstrated ($P < 0.05$). The number of apoptotic cells did not significantly differ among the tested concentrations of piperine. As for necrosis, statistically significant difference in the frequency of necrotic cells between two highest piperine concentrations and both controls was detected ($P < 0.05$). Also, a significant difference for the necrotic cells among the highest and the lowest piperine concentrations was demonstrated ($P < 0.05$). It is noticeable that the number of cells in the processes of apoptosis and necrosis increased with an increase in the concentration of piperine. In terms of the total number of cytotoxic markers, significantly elevated frequency between the highest concentration of piperine and both, negative and positive control was observed ($P < 0.05$). The total number of cytotoxic markers did not differ significantly among the tested concentrations of piperine.

The results of piperine genotoxic effects are given in Table 6. As for individual chromosomal aberrations, no significant difference was observed in the frequency of sticky chromosomes between any of the piperine concentrations and both controls, as well as between tested piperine concentrations themselves. A significantly increased number of chromosomal bridges at two highest concentrations of piperine in comparison with the negative control was detected ($P < 0.05$). However, the frequency of cells with chromosomal bridges did not differ significantly between the tested piperine concentrations. The total number of aberrant cells differed significantly between the two highest concentrations of piperine and negative control ($P < 0.05$), while no difference against positive control was demonstrated. There was no observed significant difference in the frequency of total number of aberrant cells between the tested piperine concentrations.

Table 5
Cytotoxic effects in *Allium cepa* L. apical meristem cells treated with different concentrations of piperine ($x \pm SD$, $n = 4$)

Concentration of piperine	Mitotic index (%)	Apoptotic cells	Necrotic cells	Total number of cytotoxic markers
Negative control (H_2O)	11.15 ± 2.58^{a}	0.25 ± 0.43^{a}	n.d.^{a}	0.12 ± 0.33^{a}
Positive control (EtOH) (13.3 µL/mL)	6.70 ± 3.35^{b}	1.25 ± 0.82^{baa}	n.d.^{a}	0.62 ± 0.85^{aaa}
13.3 µL/mL	1.55 ± 1.58^{cbd}	0.75 ± 0.43^{aaa}	5.00 ± 2.96^{bac}	3.11 ± 3.23^{baaa}
6.6 µL/mL	1.90 ± 1.11^{db}	n.d.^{aba}	3.20 ± 1.46^{caa}	1.77 ± 1.93^{aa}
3.3 µL/mL	4.30 ± 2.95^{ebd}	0.50 ± 0.50^{a}	2.00 ± 0.63^{aab}	1.33 ± 0.94^{a}

Note: see Table 2.

Table 6
Genotoxic effects in *Allium cepa* L. apical meristem cells treated with different concentrations of piperine ($x \pm SD$, $n = 4$)

Concentration of piperine	Sticky chromosomes	Chromosomal bridges	Vagrants	Chromosomal laggards	Micronucleus	Nuclear bud	Binuclear cells	Total number of aberrant cells
Negative control (H_2O)	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	0.250 ± 0.433^{a}	0.031 ± 0.173^{a}
Positive control (EtOH) (13.3 µL/mL)	1.000 ± 0.707^{b}	1.250 ± 0.433^{bcda}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	0.500 ± 0.500^{a}	0.392 ± 0.617^{bcda}
13.3 µL/mL	0.750 ± 0.433^{a}	1.000 ± 0.707^{cda}	n.d.^{a}	n.d.^{a}	0.250 ± 0.433^{a}	0.500 ± 0.500^{a}	1.000 ± 0.707^{a}	0.500 ± 0.626^{cda}
6.6 µL/mL	0.500 ± 0.500^{a}	1.000 ± 0.707^{da}	n.d.^{a}	n.d.^{a}	0.250 ± 0.433^{a}	0.250 ± 0.433^{a}	0.750 ± 0.433^{a}	0.392 ± 0.556^{da}
3.3 µL/mL	0.500 ± 0.500^{a}	0.750 ± 0.433^{a}	0.250 ± 0.433^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	0.250 ± 0.433^{a}	0.250 ± 0.433^{a}

Note: see Table 2.

Discussion

Various food additives, especially chemical preservatives, have a genotoxic effect on different test systems (Pandey et al., 2014). Twarog (1983) claimed that approximately one million out of nine million people with asthma in North America may be sensitive to the effects of sulfites. In order to carry out this research, the *Allium* test was used, which is a simple cyto/genotoxicity test, which allows detection of effects of various chemical compounds on the genome of higher plants such as the *Allium cepa* L. (Mesic et al., 2021). Since 1983, when Levan first used the *Allium* test, it has become the standard material for studying the effects of various chemical pollutants on chromosomes.

Baased on our results, all three tested concentrations of potassium metabisulfite significantly reduced root growth as compared to the control group. Similarly, significant inhibition in root growth was detected between two highest piperine concentrations and negative control. As for mitotic index (MI), significant decrease after the treatment with all three tested concentrations of potassium metabisulfite, as well as after the treatment with all three tested concentrations of piperine when compared to control groups, was observed. In general, toxicity is easy to detect in inhibition of root growth, while mutagenicity correlates with chromosomal aberrations (Fiskesjö, 1985). Our results are supportive of those obtained by Kumar & Panneerselvam (2007) which demonstrated decrease in mitotic index values, and increase in the frequency of chromosomal aberrations in bulbs treated with potassium metabisulfite. Similarly, Gömürgen (2005) also found that the mitotic index decreases with an increase in the concentration of potassium metabisulfite in onion apical meristem. Dias et al. (2021) demonstrated cytotoxic effects of piperine, as a significant decrease in the mitotic index was observed. The root growth is controlled by a cell division in the active meristem zone, as well as by cell elongation, whereby these two processes represent independent events (Shishkova et al., 2007). Actually, the root growth rate is affected by the disturbance of either of these two processes (Obroucheva, 2008). Therefore, it is reasonable to assume that the decrease in *A. cepa* root growth caused by potassium metabisulfite and piperine could be a consequence of a reduced mitotic index in *A. cepa* root apical meristem.

Analysis of potassium metabisulfite cytotoxicity demonstrated a significantly increased frequency of apoptosis at the two highest concentrations of potassium metabisulfite, as well as a significantly higher number of necrotic cells at all three tested concentrations of potassium metabisulfite in comparison with negative control. It is important to highlight that the total number of cytotoxic markers significantly differed between all three tested concentrations of potassium metabisulfite and negative control. As for piperine cytotoxicity, significant difference in the frequency of apoptosis between median piperine concentration and positive control was observed. Also, statistically different frequency of necrosis between two highest piperine concentrations and both controls was observed. Regarding the total number of cytotoxic markers, statistically increased frequency between the highest piperine concentration applied and negative and positive control was detected. Apoptosis is a type of cellular death that can happen as part of physiological events, while necrotic cell death occurs in response to various exogenous insults such as trauma, infection, toxins, etc. (Rock & Kono, 2008). Recent study showed that sodium metabisulfite could exhibit toxic effects on human normal cells through apoptosis activation (Alimohammadi et al., 2021). Importantly, our results are supportive of those obtained by Jaidee et al. (2022) who demonstrated that *P. nigrum* extracts induced necrosis in SW480 cell line.

As for the genotoxic effects of potassium metabisulfite, an increased frequency of chromosome aglutination at the two highest potassium metabisulfite concentrations, as well as an increased frequency of chromosomal bridges at median potassium metabisulfite concentration, in comparison with the control group was recorded. Also, a significantly elevated number of binuclear cells was detected at the highest potassium metabisulfite concentration as compared to the control. The total number of aberrant cells significantly differed between two highest concentrations of potassium metabisulfite and negative control. Analysis of piperine genotoxicity revealed an increased frequency of chromosomal bridges at two highest concentrations of piperine in comparison with the negative control. The total number of aberrant cells was significantly different between two highest piperine concentrations and control group. Agglutination of chromosomes, which subsequently lead to formation of chromosomal bridges, may appear during the occurrence of abnormalities in anaphase, or it may be attributed to unequal translocation and/or inversion of chromosomal segments (Haq et al., 2016). Chromosome agglutination is a predictor of cell deaths, because it has an irreversible effect, even inhibiting the formation of the division spindle, and leads chromosomes to stick together, unequal distribution and the creation of anaphase bridges by the fusion of chromosomes or chromatids (Sabeen et al., 2020). Binuclear cells arise as a result of an incomplete process of cell division, more precisely completed karyokinesis with incomplete cytokinesis, as well as due to the suppression of the formation of a cell plate between cells in early telophase. Binuclear cells represent nuclear changes which clearly represent biomarkers of genotoxic effects of various pollutants (Haq et al., 2016). Our findings are in concordance to those from Gömürgen (2005), who observed a numerous chromosomal abnormalities in *A. cepa* meristem cells, such as residual chromosomes, anaphase and telophase bridges and multipolar anaphase cells, of which the most common were cells with anaphase bridges. One study demonstrated cyto/genotoxic effects of piperine due to significant increase in the chromosomal changes, pointing out at the same time that the lowest piperine concentration was not genotoxic and showed a protective effect, indicating its slight antimutagenic potential that apparently depends on the piperine concentration itself (Dias et al., 2021). Based on modern studies on animal cells, piperine has been found to have immunomodulating, antioxidant, anticancer and anti-inflammatory properties (Meghwal & Goswami, 2013). Studies on mutagenic and genotoxic, as well as studies on protective and proapoptotic effects have been conducted on different types of tumor cells, but these studies have shown contradictory results for the effects of piperine. Therefore, it is important to understand the modulatory activity and find effective concentrations that are safe for the body (Singh & Duggal, 2009). According to Abo-Zeid & Farghaly (2009), when taken orally three days before mitomycin C treatment, piperine reduced the frequency of sister chromatid exchange, as well as the number of chromosomal aberrations in mouse splenocytes and spermatocytes.

Conclusion

In experiments that involved the treatment of *Allium cepa* bulbs with different potassium metabisulfite and piperine concentrations, significant changes on genetic and cellular level were observed. The results of the present study demonstrated antiproliferative and cyto/genotoxic potential of potassium metabisulfite in onion apical meristem. Also, piperine inhibited *A. cepa* meristem cell proliferation. However, the antimutagenic potential of piperine has not been confirmed, as its ability to cause significant cellular and chromosomal damaging (cyto/genotoxicity) has been detected. It is important to highlight that further studies would be of value to strengthen these findings.

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