RESEARCH ARTICLE

Therapeutic effects of royal jelly against sodium benzoate–induced toxicity: cytotoxic, genotoxic, and biochemical assessment

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Abstract

In this study, the protective role of royal jelly (RJ) against the potential toxic effects of sodium benzoate was investigated in *Allium cepa* L. test material with physiological, genetic, and biochemical parameters. Physiological changes were evaluated by determining weight gain, rooting percentage, root length, and relative injury rate. The genetic evaluations were carried out with chromosomal abnormalities (CAs), micronucleus (MN), tail DNA formation, and mitotic index (MI) ratio parameters. The biochemical evaluations were carried out by determining lipid peroxidation and antioxidant enzyme activities by determining levels of malondialdehyde (MDA), glutathione reductase (GR), superoxide dismutase (SOD), and catalase (CAT). Further, the interaction of sodium benzoate with antioxidant enzymes was evaluated with molecular docking analysis. The antimutagenic effect of RJ was evaluated as the inhibition of chromosomal abnormalities (CAs) and tail DNA formations. A total of six groups were formed in the study. *A. cepa* L. bulbs in the control group were treated with tap water; the bulbs in the administration groups were treated with sodium benzoate (100 mg/L), RJ (25 mg/L and 50 mg/L doses), and sodium benzoate–RJ combinations with these doses for 72 h. As a result, it was determined that sodium benzoate application caused inhibition of physiological parameters and MI; induced MN, CAs, and DNA damage; and also caused oxidative stress. Depending on the concentration of RJ application, it reduced sodium benzoate toxicity by showing therapeutic effects in all these parameters. Also, the interaction of sodium benzoate with antioxidant enzyme residues was determined by molecular docking analysis. As a result, it has been understood that abandoning the use of sodium benzoate will be beneficial for the environment and human health and concluded that the use of RJ in the daily diet will be effective in reducing the impact of exposed toxic ingredients.

Keywords Sodium benzoate · Micronucleus · Comet assay · Chromosomal abnormalities · Molecular docking · Lipid peroxidation · Antioxidant enzymes

Introduction

Human beings are subjected to a wide variety of xenobiotics depending on lifestyle, diet, habits, and medical purposes and for many other reasons, along with environmental and professional reasons. Therefore, the effects of xenobiotic exposure should also be investigated. An important part of xenobiotics is taken into the human body due to the use of different additives in the processing and production in the industrialized food sector (Croom 2012). During the production, processing, packaging, and storage of food, the taste, appearance, structure, smell, and other features of the food can be preserved with food additives. Additives may differ as colorants, sweeteners, and various additives depending on the application purpose (Calvo-Flores et al. 2018; Saad et al. 2005). Besides increasing flavor, food additives are used for purposes such as improving packaging and storage conditions and maintaining appearance. Intensive food additive use is assumed to be a high risk for health in the community (Bearth et al. 2014).

Sodium benzoate is used as an antimicrobial additive for foods (Clipley 2020). Sodium benzoate, the sodium salt of benzoic acid, is a pure, colorless, or crystalline powder compound with a molecular weight of 144.11 g/mol. Benzoates are used extensively in the preservation of acidic and easily acidified foods, such as carbonated drinks, cider, juices, sauerkraut, and pickles (Burdock 1997). It is also used in soft drinks, sauces, seafood, creams, toothpaste, lotions, edible

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coatings, and some curative products (Andrade et al. 2020). It has been certified by the FDA as "generally regarded as safe," which means it can be found in concentrations above 0.1% in foods (Lennerz et al. 2015). It is used in the pharmaceutical industry for the treatment of liver diseases, urea cycle disorder, and multiple sclerosis (Yadav et al. 2016). It is commonly reflected as the most active against bacteria and yeast and less active against mold (Saad et al. 2005). It has been reported that when sodium benzoate is metabolized by living organisms, an active compound may be formed that acts on DNA, affecting the genetic structure of cells and cell division (Shahmohammadi et al. 2016).

Royal jelly (RJ) is one of the most impressive foods in the dietetic and cosmetic industries. RJ is a milky and thick excretion secreted from the mandibular and hypopharyngeal glands of bees, used to feed the larvae (Isidorov et al. 2009). It includes different organic acids, amino acids, proteins, steroids, phenols, minerals, esters, sugars, trace elements, and additional components (Ramadan and Al-Ghamdi 2012). Also, RJ composition varies depending on regional and seasonal situations. Chemically, the fresh form contains water, carbohydrates, fat, proteins, mineral salts, and a small number of polyphenols and contains vitamins (Ramadan and Al-Ghamdi 2012). It has been reported that RJ contains 8 essential amino acids (isoleucine, lysine, methionine, tryptophan, leucine, threonine, valine, and phenylalanine) and 5 nonidentified related compounds (Barnutiu et al. 2011) and a small amount of vitamin C; phosphates such as ATP, ADP, and AMP; and nucleotides as free bases (Xue et al. 2017). Almost about 185 organic compounds have been identified in RJ. One of the most important proteins in RJ is Royalactin. It also includes 10-hydroxy-2-decenoic acid, an immunomodulatory fatty acid, and bioactive compounds (Sugiyama et al. 2012). It is also known that RJ is a natural antimicrobial. RJ has been reported to inhibit bacteria, especially gram-positive bacteria, and show antibacterial activity against different pathogenic bacteria (Stocker 2003). It has many biological activities, especially antimicrobial, antioxidative, immunomodulatory, tumoricidal, and anti-inflammatory activities. It is commercially available in many countries in cosmetics, healthy nutrition products, and medicines (Nakajima et al. 2009).

One of the suitable plant test models is *Allium cepa* L. used to investigate the potentially toxic effects of chemical compounds (Bhat et al. 2019). The fact that 76% of 148 chemicals whose toxicity was evaluated with the *A. cepa* L. test gave true-positive results enabled this test to be known as a standard test to determine the chromosomal abnormalities caused by the chemicals (Grant 1982). The use of *A. cepa* L., a high-level organism, as a test material provides information about potential damage to the DNA of an organism with a detoxification mechanism, unlike cell cultures (Bonciu et al. 2018; Bystrzejewska-Piotrowska and Urban 2004). The data

obtained as a result of the *Allium cepa* L. test provide accurate estimates of the effects of the agent investigated on other living biodiversity (Vicentini et al. 2001). The similarity between the findings of the toxicity experiments with the *A. cepa* L. test obtained from in vivo animal tests and in vitro cell culture tests indicates the authenticity of this test. For example, it has been reported that paraquat causes micronucleus (MN) formation in African sharptooth catfish (Oladokun et al. 2020) and *A. cepa* L. (Acar et al. 2015)., and imazalil has genotoxic effects in *A. cepa* L. (Çıldır and Liman 2020) and cultured human lymphocyte cell (Turkez and Aydın 2013). The results of the studies evaluated with the *A. cepa* L. test were compatible with other researches, indicating the reliability of the test. *A. cepa* L. test has been also used to determine the impact of environmental contaminants and chemical compounds such as water pollutants (Rodrigues et al. 2020), pesticides (Fioresi et al. 2020), wastewater (Babatunde et al. 2016), and food additives (Acar et al. 2020).

The lack of a systematic research on sodium benzoate toxicity and the therapeutic effects of the RJ led to the notion of examining both effects in vivo with this study. In this research, toxic effects of sodium benzoate and the therapeutic role of RJ were investigated on *Allium cepa* L. test material with the physiological, genetic, and biochemical parameters.

Materials and methods

Test material

Allium cepa L. bulbs used for testing were purchased from the local market, and plant material was approved by using taxonomic characters at Giresun University, Faculty of Arts and Sciences, Botany Department.

Preparation of royal jelly

Royal jelly (RJ) was acquired from a local beekeeper in Giresun, Turkey. RJ collected from the 3-day-old larvae of the queen bees was kept frozen at −20°C until used. It was diluted in distilled water and shaken gently overnight. Appropriate doses were prepared for the test by diluting the supernatant.

Determination of application doses

Although there is no study on the effective dose of sodium benzoate in plant test materials, other studies in the literature were examined and the appropriate dose was determined. The EC_{50} value of sodium benzoate against chymotrypsin and trypsin activity has been reported as 25 mg/L and 80 mg/L, respectively (Esimbekova et al. 2017). The European Food Safety Authority (EFSA 2016) reported the LD_{50} level for

sodium benzoate as 2000 mg/kg. Doses of 1, 1.5, and 2 mg/mL (Pongsavee 2015) and 6.25, 12.5, 25, 50, and 100 μg/mL (Zengin et al. 2011) were administered in genotoxicity studies performed on lymphocyte cells. Due to the effective doses used in the studies and the reported high EC_{50} and LD_{50} values, the lower dose of 100 mg/L was preferred in the study and thus, the effect of sodium benzoate at low doses could be observed.

The protective role of royal jelly on plant test material has not been studied much, and there is no evidence in the literature that it has a toxic effect. Therefore, the selection of royal jelly doses used in the study was made depending on the doses used in previous studies (Türkmen et al. 2009; Tohamy et al. 2019; Qiu et al. 2020; Waykar and Alqadhi 2020). It was determined that the lowest doses applied in the studies examined were 25 mg/L and 50 mg/L, and these doses were preferred in this study. Thus, the effect of royal jelly at low doses will be observed.

Experimental design

Six groups were formed, containing approximately equally sized and healthy *A. cepa* L. bulbs, with 50 bulbs in each group. The control group was treated with tap water, and application groups were treated with sodium benzoate (100 mg/L), RJ (25 mg/L and 50 mg/L), and their combination at 24 °C during 72 h. The application was made in 60 × 42-mm beakers containing 40-mL application solution for each bulb, and the roots were directly exposed to the application solutions. Fifty bulbs for each group were used for the macroscopic analysis of physiological parameters, and 10 bulbs were randomly selected in each group for microscopic and biochemical analysis. Application groups and treatments are given in Table 1.

Measurement of physiological parameters

The weight gain of *A. cepa* L. bulbs was determined by measurements made with sensitive scales before and after application. Root lengths were measured with a millimetric ruler on the basis of radicle formation. The percentage of rooting and relative injury rates were calculated by using Eqs. 1 (Atik et al. 2007) and 2 (Praveen and Gupta 2018).

$$\text{Rooting percentage (\%)} = \frac{\text{Rooted bulbs}}{\text{Total number of bulbs}} \quad (1)$$

Relative injury rate

$$= \frac{\text{\%Rooting percentage in control} - \text{\%Rooting percentage in each group}}{\text{\%Rooting percentage in control}} \quad (2)$$

Determination of chromosomal abnormalities, micronucleus, and mitotic index

One to 2 cm of samples from the root tips was fixed for 2 h in Clarke solution (ethanol, glacial acetic acid, 3:1) and held for 15 min in 96% ethanol. For analysis procedures, samples were processed in 70% ethanol at 4 °C. For micronucleus and mitotic index (MI) analysis, the roots were handled with 1N HCl for 17 min at the temperature of 60 °C, incubated with 45% acetic acid for 30 minutes, and stained for 24 h in acetocarmine. Preparations of mitotic cells were analyzed and counted under a research microscope at ×500 magnification (Staykova et al. 2005). The protocol of the Fenech et al. (2003) evaluation for the involvement of MN has been carried out.

The preparations were examined under a binocular research microscope to determine the MI, and the MI percentage was determined using Eq. 3.

$$\text{Mitotic Index (\%)} = \frac{\text{Number of mitotic cells}}{\text{Total number of cells}} \quad (3)$$

For each group, 10 slides were prepared from the root tips taken from randomly selected bulbs, and 1000 cells for MN frequency and 10000 cells for MI were counted in each slide.

Comet assay

Comet assay (alkaline single-cell gel electrophoresis) was performed according to the protocol of Chakraborty et al. (2009) with slight modifications. Roots taken randomly from each group were quickly crushed with a raster tool in 400 μL of Tris buffer (cold, 0.4 M, pH 7.5) and a mixture of 1:1 1% low melting point agarose (LMPA) and nuclear suspension in phosphate-buffered saline (PBS) on pre-coated sheets. The coverslip was covered at 40 °C with 1% normal melting point agarose (NMPA). After completing the gelling step of the LMPA, the coverslip was slowly removed and slides were moved to a horizontal gel electrophoresis tank containing fresh and chilled electrophoresis buffer. Electrophoresis was

Table 1 Application groups

Groups	Application
Group I	Tap water
Group II	25 mg/L royal jelly
Group III	50 mg/L royal jelly
Group IV	100 mg/L sodium benzoate
Group V	100 mg/L sodium benzoate + 25 mg/L royal jelly
Group VI	100 mg/L sodium benzoate + 50 mg/L royal jelly

performed at 0.7 V/cm at 4 °C (20 V, 300 mA) for 15 min using a power supply. Slides were rinsed three times with filtered water and neutralized with Tris buffer (0.4 M Tris, pH 7.5). The nuclei were stained for 5 min with ethidium bromide after immersion in cold water for 5 min. The preparations were washed with cold water and eliminated residual stain and coverslip sealed. These steps were taken with low light to avoid DNA degradation and examined with a fluorescence microscope. Comets were analyzed with Comet Assay Software version 1.2.3b (Końca et al. 2003). For comet assay, 10 slides were prepared from the root tips taken from randomly selected bulbs in each group and 500 cells per slide were analyzed, which were used for the percentage of tail DNA, tail moment, and olive tail moment.

Evaluation of antimutagenic effects

The antimutagenic influence of RJ was calculated by Eq. 4 (Acar 2021). In order to assess the antimutagenic effect, chromosomal abnormalities (CAs) and the percentage of tail DNA were used.

$$\text{Mutagenicity inhibition (\%)} = \frac{\text{Sodium benzoate group damage (\%)} - \text{Sodium benzoate with RJ group damage (\%)}}{\text{Sodium benzoate group damage (\%)} - \text{Control group damage (\%)}} \times 100 \qquad (4)$$

Lipid peroxidation

Lipid peroxidation was evaluated by measuring the amount of MDA according to the protocol of Ünyayar et al. (2006). Approximately 0.5 g of root tissue was split into small sections and homogenized with 5% trichloroacetic acid (TCA), and the homogenates were centrifuged at 12,000 rpm at 24 °C for 15 min. The supernatant, TCA solution (20%), and thiobarbituric acid (0.5%) have been transferred to the new tube and incubated at 96 °C for 25 min. The tubes were taken into the ice bath and centrifuged at 10,000 rpm for 5 min. The absorbance was estimated at 532 nm, the extinction coefficient which was 155 mM/cm has been used to determine the quantity of MDA content, and levels were taken from measurements of three independent samples and given as the mean amount of MDA ± standard error (SE).

Antioxidant enzyme assays

Superoxide dismutase

The SOD level was calculated according to the protocol of Beauchamp and Fridovich (1971). 0.5 g of root material was homogenized in 5 mL of 50 mM (pH 7.8) chilled sodium phosphate buffer. The homogenates were centrifuged at 10,500 rpm for 20 min, and the supernatant was used for the analysis of enzymes. The reaction mixture containing 0.3 mL 130 mM methionine, 1.5 mL 0.05 M sodium phosphate buffer (pH 7.8), 0.3 mL 0.1 mM EDTA-Na$_2$, 0.3 mL 750 µM nitroblue tetrazolium chloride (NBT), 0.3 mL of 20 µM riboflavin, 0.01 mL of 4% (w/v) insoluble polyvinylpyrrolidone, 0.01 mL of enzyme extract, and 0.28 mL of deionized water was prepared. The reaction began with putting the tubes under two 15-W fluorescent lamps for 10 min and ending by keeping the tubes in the dark for 10 min. Absorbance was measured at 560 nm, and a unit SOD enzyme activity was determined as the amount of SOD enzyme required for 50% inhibition of NBT reduction under application conditions. SOD levels were taken from the measurements of three independent samples and expressed as mean units per milligram fresh weight (FW) ± standard error (SE) (U/mg FW).

Catalase

Catalase (CAT) activity was determined by the protocol of Beers and Sizer (1952). CAT activity was determined by a UV-VIS spectrophotometer in a reaction mixture of 2.8 mL of 0.3 mL 0.1 M H$_2$O$_2$, 1 mL deionized water, and 200 mM sodium phosphate (1.5 mL) formulated just before use to evaluate the reaction combination. The reaction was triggered by adding 0.2 mL of supernatant, and CAT activity was measured by monitoring the absorbance decrease (240 nm) as a result of H$_2$O$_2$ consumption. Units of CAT activity were determined by units per minute per gram fresh weight; one unit of CAT activity was defined for a change of 0.1 at an absorbance of 240 nm, and values are taken from the measurements of three independent samples and expressed as mean ± standard error (SE) OD$_{240nm}$/minute·gram FW.

Glutathione reductase

Glutathione reductase (GR) levels were determined by making slight modifications in the protocol defined by Carlberg and Mannervik (1975). Shortly, the root tips (0.5 g) in 0.2 M EDTA (pH 4.7) were homogenized. The GR level was measured in a 2-mL reaction mixture containing 1 M oxidized glutathione (GSSG), 0.1 mM nicotinamide adenine dinucleotide phosphate (NADPH), 0.05 M potassium phosphate buffer (pH 7.0), and 3 mM EDTA. The supernatant absorbances were recorded at 340 nm and values are taken from the measurements of three independent samples. The levels of GR

were expressed as mean micromole NADPH/minute·gram FW ± standard error (SE).

Molecular docking

Molecular docking was performed to analyze the interactions of the antioxidant enzymes catalase, superoxide dismutase, and glutathione reductase with sodium benzoate. The crystallographic 3D structure of the SOD (PDB ID: 1ba9) (Banci et al. 1998), CAT (PDB ID: 5gkn) (Yonekura and Maki-Yonekura 2016), and GR (PDB ID: 2hqm) (Yu and Zhou 2007) was obtained from the protein data bank. The 3D structure of sodium benzoate (PubChem CID: 517055) was retrieved from the PubChem. Proteins were prepared using Biovia Discovery Studio 2020 Client for docking. For the docking process, in crystallographic structures, model 1 was used for SOD and chain A was used in CAT and GR structure because there is more than one of the same chain structure. In the preparation process, the active site was determined; after removing the water molecules and co-crystal ligands, polar hydrogen was added. Energy minimization was done with

Gromos 43B1 using Swiss-PdbViewer (Guex and Peitsch 2005) (v.4.1.0) software for proteins; ligand energy minimization was done with the uff-force field using Open Babel v.2.4.0 software (O'Boyle et al. 2011). The molecular docking process was carried out with the grid box to contain the active areas. Molecular docking was done with AutoDock Vina software (Trott and Olson 2010). The docking analyses and 3D visualizations were performed with Biovia Discovery Studio 2020 Client.

Dose-response relationship of royal jelly

The evaluation of the dose-response relationship of RJ against sodium benzoate toxicity was performed by calculating the percentage healing effect of RJ against the changes caused by sodium benzoate toxicity in all parameters. The healing percentage caused by RJ in the calculation was calculated by proportioning with the sodium benzoate application group and control group data. For this, Eq. 5 was used and evaluated with the logarithmic values of the doses.

$$\text{Recovery effect of RJ (\%)} = \frac{\text{Sodium benzoate with RJ group parameter} - \text{Sodium benzoate group parameter}}{\text{Sodium benzoate group parameter} - \text{Control group parameter}} \times 100 \qquad (5)$$

Statistical analysis

SPSS Statistics v23.0 (IBM Corp. USA) package program was used to perform statistical analyses. Data were expressed as mean ± SD (standard deviation) in the tables and mean ± SEM (standard error of means) in the graphs. The statistical significance between the means was determined by the method of one-way ANOVA and Duncan's test, and the $P < 0.05$ was deemed statistically significant.

Results

Physiological parameters

The physiological effects of sodium benzoate application on *A. cepa* L. test material and the protective role of RJ against these effects are given in Table 2. The highest rooting percentage, root length increase, and weight gain were observed in group I, which was the control group, and in group II and group III, where RJ doses were applied at 25 mg/L and 50 mg/L, respectively. It was determined that the changes in physiological parameters in these three groups were not statistically significant ($P > 0.05$). Application of 100 mg/L dose of sodium benzoate alone (group IV) caused dramatic

decreases in rooting percentage, root length, and weight. Sodium benzoate application caused a reduction in root length of *A. cepa* L test material. After 72 h of treatment, the mean root length was 14.73 cm in the control group, and 5.13 cm in group IV. In other words, administration of sodium benzoate alone at a dose of 100 mg/L reduced root length 65.17% compared to the control, and this decrease was statistically significant ($P < 0.05$). Application of sodium benzoate alone caused significant decreases in rooting percentage and weight gain parameters. In the control group (group I), rooting percentage was determined as 96% and the average weight gain was 9.11 g. In group IV, the rooting percentage was determined as 42% and weight gain was determined as 2.21 g. These changes were statistically significant ($P < 0.05$). All these parameters showed that sodium benzoate administration inhibits the increase in physiological parameters by showing a toxic effect on *A. cepa* L. test material. In addition to the application of sodium benzoate at a dose of 100 mg/L, in group V and group VI, in which 25 mg/L and 50 mg/L doses of RJ were applied respectively, an increase occurred again in physiological parameters depending on the dose of RJ applied. Compared to group IV, 25 mg/L RJ dose application in combination with sodium benzoate in group V increased the rooting percentage by 56%, the mean root length by 59.26%, and the weight gain by 102.27%. In group VI, 50 mg/L royal jelly application in combination with sodium

Table 2 Effect of sodium benzoate and RJ on physiological parameters

Groups	Rooting percentage (%)	Mean root length	Relative injury rate	Initial weight	Final weight	Weight gain (g)
Group I	98	14.73 ± 2.71^c	-	10.24 ± 1.12^e	19.35 ± 1.93^a	9.11
Group II	100	15.31 ± 2.98^b	-	9.81 ± 1.21^e	19.06 ± 2.62^a	9.25
Group III	100	15.97 ± 3.02^a	-	9.98 ± 1.16^e	20.12 ± 2.80^a	10.15
Group IV	42	5.13 ± 2.07^f	0.56	9.91 ± 1.50^e	12.12 ± 1.89^d	2.21
Group V	56	8.17 ± 2.29^e	0.42	9.69 ± 1.45^e	14.14 ± 1.86^c	4.45
Group VI	70	10.82 ± 3.14^d	0.28	9.74 ± 1.19^e	16.10 ± 1.89^b	6.36

Group I: tap water (control), group II: 25 mg/L RJ, group III: 50 mg/L RJ, group IV: 100 mg/L sodium benzoate, group V: 100 mg/L sodium benzoate + 25 mg/L RJ, group VI: 100 mg/L sodium benzoate + 50 mg/L RJ. Data are shown as mean ± standard deviation (SD). The averages shown with different letters in the same column are statistically significant ($P < 0.05$)

benzoate increased the rooting percentage by 70%, the average root length by 110.92%, and the weight gain by 189.09% compared to group IV. Application of RJ inhibited the toxicity of sodium benzoate in physiological parameters depending on the application dose and showed an improvement in all these parameters. It was determined that the increases caused by RJ in the physiological parameter values depending on the application dose were statistically significant ($P < 0.05$). However, none of the findings were able to reach the values in groups I–III. RJ application caused a dose-related decrease in the relative injury rate, which is the damage determination parameter. Although the relative injury rate was 0.56 in group IV, this value decreased by 25% in group V to 0.46 and decreased by 50% in group VI to 0.28. This reduction occurred due to the applied RJ dose.

Chromosomal abnormalities, micronucleus, and mitotic index

The cytogenetic effects of sodium benzoate administration and the protective role of RJ were investigated by the percentage of MI, MN formation, and CA frequency. The effects of sodium benzoate on MN formation (Fig. 1a) and MI and the protective role of RJ against these effects are given in Table 3. Only a few MN formation was observed in the control group (group I), and there was no MN formation detected in group II and group III, which were administered 25 mg/L and 50 mg/L doses of RJ, respectively. There was no statistically significant difference between these three groups in MN formation ($P > 0.05$). In group IV, where 100 mg/L dose of sodium benzoate was applied alone, and MN formation was detected at the frequency of 41.60, the highest value among the groups. In group V and group VI, where 25 mg/L and 50 mg/L doses of RJ were applied in combination with sodium benzoate, respectively, the rate of MN formation decreased 17.79% to an average of 34.20 and decreased 43.27% to an average of 23.60, compared to group IV, respectively.

The effect of sodium benzoate and RJ applications on cell division was evaluated by determining the mitotic index rates.

The MI frequency was determined as 952.40 in the control group (group I), 959.60 in group II (25 mg/L RJ + 100 mg/L sodium benzoate), and 967.70 in group III (50 mg/L RJ + 100 mg/L sodium benzoate). It was determined that the differences in MI frequency calculated between these three groups were not statistically significant ($P > 0.05$). There was a dramatic decrease in MI frequency in group IV, where sodium benzoate was applied alone, and the lowest MI frequency was observed in this group. In group IV and group VI, where 25 mg/L and 50 mg/L RJ were administered in combination with sodium benzoate (100 mg/L), the MI frequency was 620.90 and 706.80, respectively. Sodium benzoate application showed toxic effect by inhibiting cell division; RJ application showed therapeutic effect by inhibiting this toxicity depending on the dose.

Chromosomal abnormalities caused by sodium benzoate application in *A. cepa* L. root tip cells are given in Table 4 and Fig. 1. There were no chromosomal abnormalities in group I, except for a few fragment formation, and no chromosomal abnormality was found in groups II and III treated with RJ. The highest frequency of chromosomal abnormalities was detected as a rate of 200.10 ± 19.86 in group IV, which is the 100 mg/L dose of sodium benzoate–alone administration group, and the chromosomal abnormalities occurring are fragment, sticky chromosome, unequal distribution of chromatin, bridge, binucleated cell, and vagrant chromosome in order of frequency. In group V and group VI, where 25 mg/L and 50 mg/L doses of RJ were applied in combination with sodium benzoate, respectively, a decrease occurred in the frequencies of all these chromosomal abnormalities and the total frequency of chromosomal abnormalities was found to be 134.70 ± 18.26 and 93.20 ± 12.56, respectively. These decreases in chromosomal abnormalities show that RJ has a dose-dependent antimutagenic effect and reduces sodium benzoate–induced mutagenicity. The antimutagenic effect was also determined by the percentage of mutagenicity inhibition. According to the calculations made according to the total of chromosomal abnormalities in group IV, mutagenicity inhibition was determined as 32.77% in group V, where 25
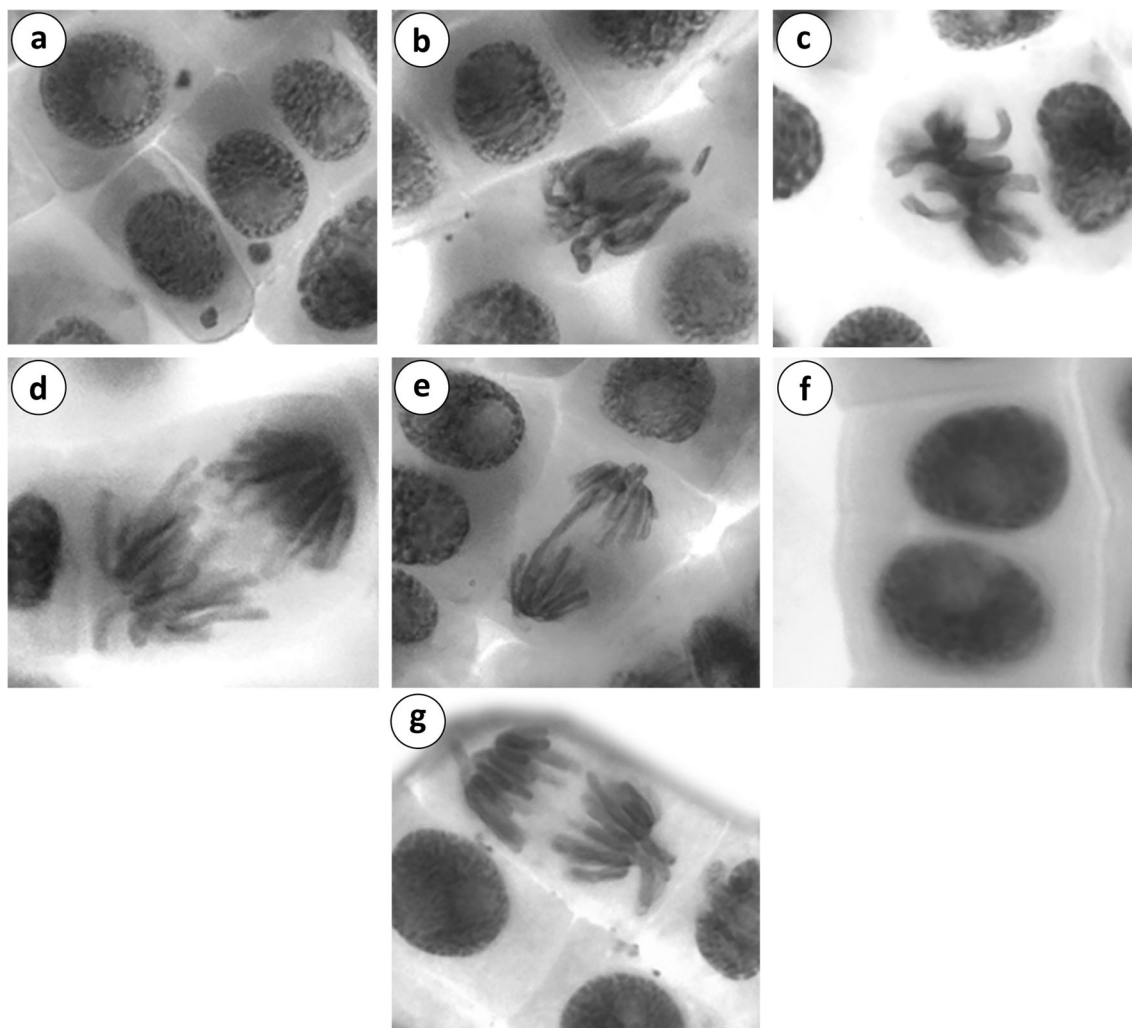
Fig. 1 Chromosomal abnormalities and MN formations caused by sodium benzoate. **a** MN. **b** Fragment. **c** Sticky chromosome. **d** Unequal distribution of chromatin. **e** Bridge. **f** Binucleated cell. **g** Vagrant chromosome

mg/L dose of RJ was applied, and 53.56% in group VI, where 50 mg/L dose of RJ was applied. These findings show that RJ

Table 3 Effects of sodium benzoate and RJ application on MN formation and mitotic index (MI)

Groups	MN formation	Mitotic index (MI)	Mitotic index rate (%)
Group I	0.40 ± 0.70[d]	952.40 ± 23.35[a]	9.52
Group II	0.00 ± 0.00[d]	959.60 ± 26.09[a]	9.60
Group III	0.00 ± 0.00[d]	967.70 ± 18.04[a]	9.68
Group IV	41.60 ± 9.56[a]	539.50 ± 32.88[d]	5.40
Group V	34.20 ± 8.20[b]	620.90 ± 39.33[c]	6.21
Group VI	23.60 ± 4.93[c]	706.80 ± 37.48[b]	7.07

Group I: tap water (control), group II: 25 mg/L RJ, group III: 50 mg/L RJ, group IV: 100 mg/L sodium benzoate, group V: 100 mg/L sodium benzoate + 25 mg/L RJ, group VI: 100 mg/L sodium benzoate + 50 mg/L RJ. Data are shown as mean ± standard deviation (SD). The averages shown with different letters in the same column are statistically significant ($P <$ 0.05)

shows an antimutagenic effect depending on the dose and leads to improvement in all these cytogenetic parameters.

Comet assay

DNA damage caused by sodium benzoate in the cell nuclei of *A. cepa* L. and the protective role of RJ were evaluated using single-cell gel electrophoresis with the percentage of tail DNA, tail moment, and olive tail moment. The effects of sodium benzoate and RJ applications on DNA in *A. cepa* L. root tip cells are given in Fig. 2 and Table 5. There was no statistically significant difference ($P >$ 0.05) in tail DNA percentage, tail moment, and olive tail moment formation between the control group (group I) and RJ-alone application groups (groups II and III). In group IV, where sodium benzoate (100 mg/L) was applied alone, the tail DNA percentage was 49.43, tail moment 45.35, and olive tail moment 29.26, and these values indicate that the most DNA damage occurred in this group. In group V (25 mg/L RJ) and group VI (50 mg/L

Table 4 Frequencies of chromosomal abnormalities caused by sodium benzoate and the genoprotective role of RJ

	Group I	Group II	Group II	Group IV	Group V	Group VI
FRG	0.50 ± 0.85^d	0.00 ± 0.00^d	0.00 ± 0.00^d	67.90 ± 12.99^a	41.90 ± 10.17^b	33.10 ± 7.50^c
SC	0.00 ± 0.00^d	0.00 ± 0.00^d	0.00 ± 0.00^d	40.30 ± 13.28^a	28.20 ± 7.16^b	19.90 ± 9.31^c
UDC	0.00 ± 0.00^d	0.00 ± 0.00^d	0.00 ± 0.00^d	32.90 ± 9.39^a	22.40 ± 6.90^b	14.60 ± 4.27^c
B	0.00 ± 0.00^d	0.00 ± 0.00^d	0.00 ± 0.00^d	23.60 ± 9.94^a	16.30 ± 6.88^b	11.20 ± 5.43^c
BC	0.00 ± 0.00^d	0.00 ± 0.00^d	0.00 ± 0.00^d	19.60 ± 7.49^a	14.30 ± 4.99^b	8.10 ± 4.46^c
VC	0.00 ± 0.00^d	0.00 ± 0.00^d	0.00 ± 0.00^d	15.80 ± 6.16^a	11.60 ± 3.84^b	6.30 ± 2.63^c
Total CAs	0.50 ± 0.85^d	0.00 ± 0.00^d	0.00 ± 0.00^d	200.10 ± 19.86^a	134.70 ± 18.26^b	93.20 ± 12.56^c
CAs (%)	0.05	0	0	20.01	13.47	9.32
Mutagenicity inhibition (%)	-	-	-	-	32.77	53.56

Group I: tap water (control), group II: 25 mg/L RJ, group III: 50 mg/L RJ, group IV: 100 mg/L sodium benzoate, group V: 100 mg/L sodium benzoate + 25 mg/L RJ, group VI: 100 mg/L sodium benzoate + 50 mg/L RJ. Data are shown as mean ± standard deviation (SD). The averages shown with different letters in the same line are statistically significant ($P < 0.05$). *FRG* fragment, *SC* sticky chromosome, *UDC* unequal distribution of chromatin, *B* bridge, *BC* binucleated cell, *VC* vagrant chromosome

RJ), where different doses of RJ were administered in combination with sodium benzoate, the percentage of tail DNA was 30.29 and 22.18, tail moment was 20.45 and 11.03, and the olive tail moment was 14.50 and 9.36, respectively. Depending on the application dose, RJ decreased sodium benzoate–induced DNA damage and induced a decrease in the percentage of tail DNA, tail moment, and olive tail moment. The antimutagenic effect was assessed by the reduction in the percentage of tail DNA, and the antimutagenic effect of RJ applications against sodium benzoate genotoxicity was determined as 41.22% for 25 mg/L (group V) and 57.33% for 50 mg/L (group VI). The cause of DNA damage may be due to oxidative stress caused by sodium benzoate, and the reduction in damage may be due to the interaction of RJ application with DNA repair processes.

Lipid peroxidation and antioxidant enzymes

The effects of sodium benzoate and RJ on lipid peroxidation were determined by the determination of the MDA level, and the effects on oxidative stress by the activities of the GR, SOD, and CAT antioxidant enzymes. The effects of sodium benzoate and RJ applications on MDA levels are given in Fig. 3a. Average levels of MDA were 6.75 μmol/g FW in group I, 6.82 μmol/g FW in group II, and 6.84 μmol/g FW in group III. It was determined that there was no statistically significant difference between the measured MDA values in these groups ($P > 0.05$). In group IV, where sodium benzoate was applied alone at a dose of 100 mg/L, the MDA level increased by approximately 343.26% to 29.92 μmol/g FW compared to the control group (group I). This was the highest level of MDA observed among the groups. In group V and group VI, where 25 mg/L and 50 mg/L doses of RJ were administered in combination with sodium benzoate, respectively, MDA levels were determined as 21.58 μmol/g FW and 17.41 μmol/g FW, respectively, and decreasing by 27.87% and 41.81% compared to group IV. In other words, sodium benzoate application caused a sharp increase in MDA level, and royal jelly applied in combination with sodium

Fig. 2 Comet assay in *A. cepa* L. root tip cell nuclei. **a** Control. **b** 25 mg/L RJ. **c** 50 mg/L RJ. **d** 100 mg/L sodium benzoate. **e** 100 mg/L sodium benzoate + 25 mg/L RJ. **f** 100 mg/L sodium benzoate + 50 mg/L RJ
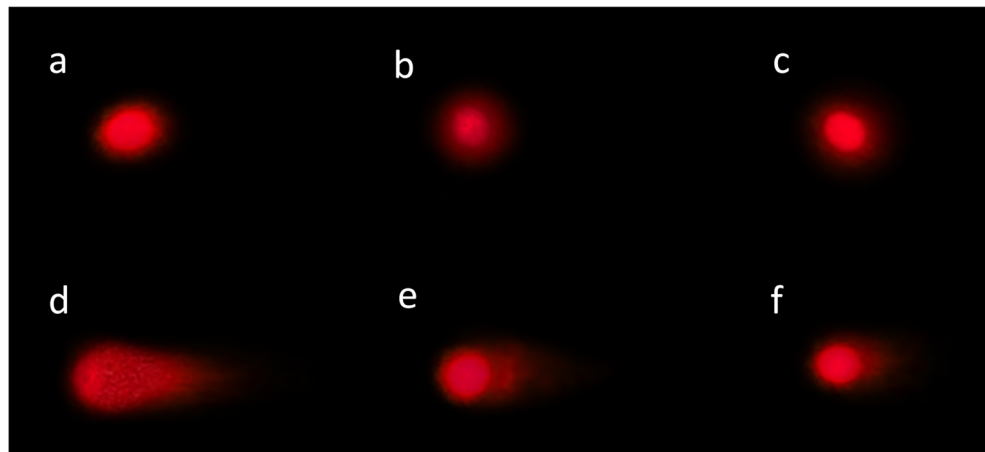
Table 5 Detection of the effects of sodium benzoate and RJ application on DNA by comet test

Groups	Head DNA (%)	Tail DNA (%)	Tail moment	Olive tail moment	Mutagenicity inhibition (%)
Group I	96.12 ± 1.95[a]	3.88 ± 1.44[d]	0.81 ± 0.28[d]	1.62 ± 0.20[d]	-
Group II	97.75 ± 2.03[a]	2.25 ± 2.03[d]	0.64 ± 0.21[d]	1.55 ± 0.45[d]	-
Group III	98.04 ± 2.39[a]	1.96 ± 2.39[d]	0.59 ± 0.16[d]	0.94 ± 0.50[d]	-
Group IV	50.57 ± 9.03[d]	49.43 ± 9.03[a]	45.35 ± 8.06[a]	29.26 ± 6.45[a]	-
Group V	69.71 ± 5.85[c]	30.29 ± 5.85[b]	20.45 ± 7.25[b]	14.50 ± 6.02[b]	42.02
Group VI	77.82 ± 6.39[b]	22.18 ± 6.39[c]	11.03 ± 3.25[c]	9.36 ± 3.05[c]	59.82

Group I: tap water (control), group II: 25 mg/L RJ, group III: 50 mg/L RJ, group IV: 100 mg/L sodium benzoate, group V: 100 mg/L sodium benzoate + 25 mg/L RJ, group VI: 100 mg/L sodium benzoate + 50 mg/L RJ. Data are shown as mean ± standard deviation (SD). The averages shown with different letters in the same column are statistically significant ($P < 0.05$)

benzoate inhibited this increase and caused improvement depending on the application dose.

The effects of sodium benzoate and RJ on GR levels are given in Fig. 3b. The average GR level in group I (control) was 6.82 μmol NADPH/min·g FW, and in group II and group III where RJ was applied alone (25 and 50 mg/L), it was found to be 6.96 μmol NADPH/min·g FW and 6.78 μmol NADPH/ min·g FW, respectively. It was determined that the difference between these groups was not statistically significant ($P > 0.05$). In group IV, where sodium benzoate was applied alone, the GR level increased dramatically and was found to be 11.56 μmol NADPH/min·g FW. In other words, sodium

benzoate application caused a sharp increase in the GR level. In group V and group VI, administration of 25 mg/L and 50 mg/L doses of RJ combination with sodium benzoate caused a dose-dependent decrease in GR level, and GR levels were found to be 9.25 μmol NADPH/min·g FW and 8.44 μmol NADPH/min·g FW, respectively. Therefore, RJ application had a reverse effect on the increase in GR level caused by sodium benzoate, and this effect occurred depending on the dose. It was determined that the differences in GR level occurring between these groups were statistically significant both among themselves and according to the first three groups ($P < 0.05$).
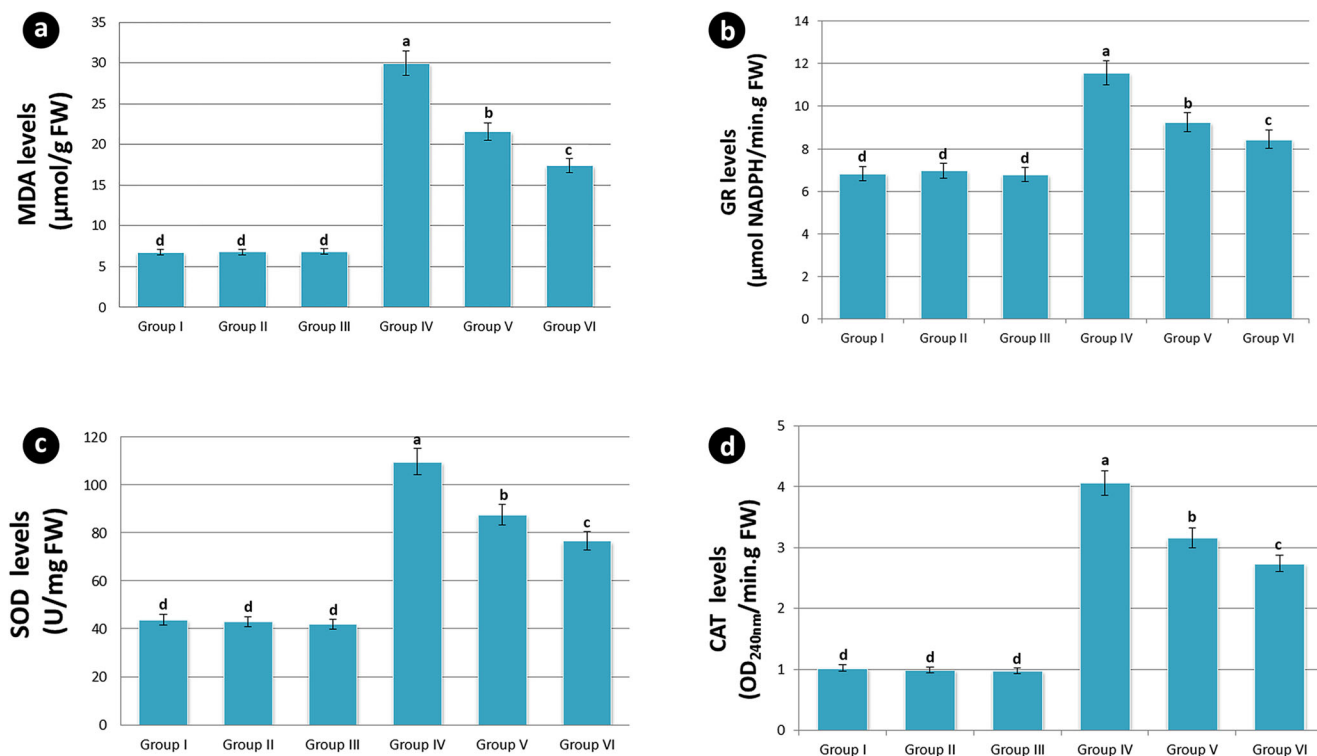

Fig. 3 Effects of sodium benzoate and RJ applications on lipid peroxidation and antioxidant enzyme levels. **a** MDA levels. **b** GR levels. **c** SOD levels. **d** CAT levels. Data were shown as mean ± SE. The averages shown with different letters in each graph are statistically significant ($P < 0.05$)

The effects of sodium benzoate and RJ applications on SOD and CAT levels are given in Fig. 3c and 3d. SOD levels in group I, group II, and group III were determined as 43.78 U/mg FW, 42.85 U/mg FW, and 41.90 U/mg FW, respectively; CAT levels were determined as 1.02 OD_{240nm}/min·g FW, 0.99 OD_{240nm}/min·g FW, and 0.97 OD_{240nm}/min·g FW. It was determined that the difference between SOD and CAT levels determined in group I (control), group II, and group III (royal jelly–alone application groups) was not statistically significant ($P > 0.05$). SOD and CAT levels increased significantly in group IV, the sodium benzoate–alone application group, and were determined to be 109.65 U/mg FW and 4.06 OD_{240nm}/min·g FW, respectively. In group V and group VI, SOD levels were determined as 87.47 U/mg FW and 76.65 U/mg FW, and CAT levels as 3.16 OD_{240nm}/min·g FW and 2.74 OD_{240nm}/min·g FW, respectively. RJ applied in combination with sodium benzoate inhibited the increase in SOD and CAT levels caused by sodium benzoate and showed a dose-dependent improvement. It was determined that the differences in SOD and CAT levels between these groups were statistically significant both among themselves and compared to the first three groups, which were the control and only royal jelly application groups ($P < 0.05$).

Molecular interactions

The interactions between sodium benzoate and antioxidant enzymes were demonstrated by molecular docking analysis. The lowest values of the binding affinities and the lowest root mean square deviation (RMSD) scores were preferred as the best docking pose. The hydrogen bonding and hydrophobic interactions between the ligand and proteins with their binding affinities are given in Table 6 and Fig. 4. It has been understood that many interactions with hydrogen bonds and hydrophobic interactions occur between sodium benzoate and antioxidant enzymes. In all molecular docking results, docking complexes with the lowest RMSD values and the best binding affinity results were evaluated. The negative value (the lowest value) in the binding energy of each complex indicates higher free binding energy and hence the stronger interaction

probability (Purich 2010). In the sodium benzoate–SOD structural complex, it has been observed that the ASP96 and THR88 residues of SOD develop hydrogen bonds with sodium benzoate and hydrophobic interactions occurred with SER469, GLU471, LEU492, and TYR505 residues (Fig. 4a). In the sodium benzoate–CAT complex, the ASN148 residue of catalase was involved in the hydrogen bond interactions of the sodium benzoate (Fig. 4b), and the best binding affinity for this interaction was −6.0 kcal/mol. Also, in this complex, PHE197 and ARG202 participated in hydrophobic interactions. The best binding affinity for this interaction was −4.0 kcal/mol. Sodium benzoate showed hydrogen bond interactions of GR with ALA54 and GLY137 residues and hydrophobic interactions with TRP138, HIS298, and ALA54 residues (Fig. 4c). Sodium benzoate showed the highest binding affinity with GR among antioxidant enzymes and was determined to be −6.2 kcal/mol. Overall, molecular docking associations and binding energy have demonstrated that antioxidant enzymes may be susceptible to the presence of sodium benzoate. Molecular docking results showed that sodium benzoate can interact with antioxidant enzyme residues, affecting enzyme activities and functions.

Dose-dependent curative effects of RJ

The curative effects of RJ on physiological, genetic, and biochemical parameters against sodium benzoate–induced toxicity are shown in Fig. 5. The dose-response recovery curves in the graph show that RJ exhibits an enhancement effect in physiological, genetic, and biochemical parameters depending on the application dose. At the 50 mg/L dose of RJ where the best therapeutic effect was detected, it caused an improvement between 48.28 and 60.14% in physiological parameters, an improvement between 41.09 and 53.56% in genetic parameters, an improvement of 53.99% in lipid peroxidation, and an improvement in antioxidant enzyme levels between 43.42 and 50.10%. The graph showed that the therapeutic effects occurring with the administration of RJ are dose-dependent and in close range, which is another indication that the genetic and biochemical parameters in the study are directly or indirectly related.

Table 6 Binding affinities of sodium benzoate to antioxidant enzymes and interactions with amino acid residues

Antioxidant enzyme	RMSD lower bound	RMSD upper bound	Binding affinity (kcal/mol)	Hydrogen bond interactions	Hydrophobic interactions
SOD	0	0	−4	ASP96 THR88	VAL87 VAL97 ILE99
CAT	0	0	−6	ASN148	PHE197 ARG202
GR	0	0	−6.2	GLY137 ALA154	TRP138 HIS298 ALA54

Fig. 4 Molecular interactions of sodium benzoate with antioxidant enzymes. **a** Sodium benzoate–SOD complex. **b** Sodium benzoate–CAT complex. **c** Sodium benzoate–GR complex
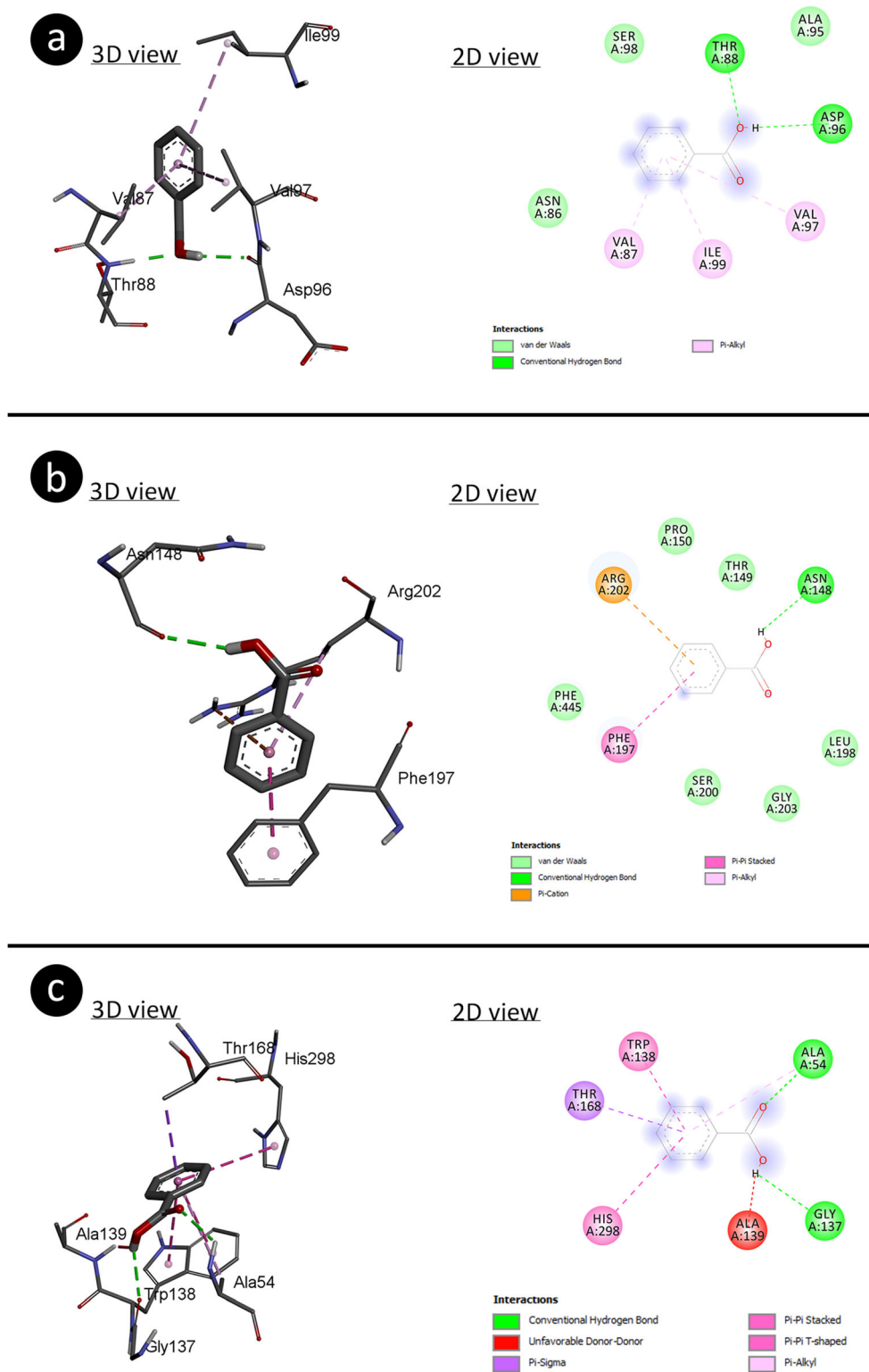
Discussion

In this study, the physiological, genetic, and biochemical effects of sodium benzoate and the protective role of RJ against these effects have been investigated. Also, the interactions of

sodium benzoate with antioxidant enzyme residues were investigated by molecular docking analysis. Sodium benzoate had an inhibitory effect on physiological parameters, and there were statistically significant reductions in rooting percentage, root length, and weight gain parameters compared to the
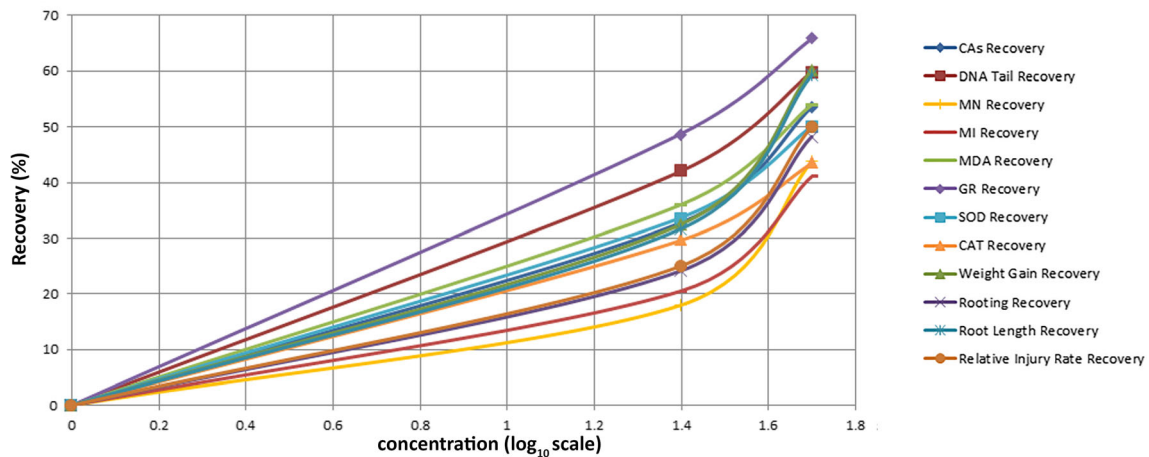
Fig. 5 Dose-response recovery curves of RJ against sodium benzoate toxicity

control group ($P < 0.05$). RJ applied in combination with sodium benzoate tolerated this decrease and caused an increment in physiological parameters depending on the dose of RJ applied. Other studies investigating the effect of sodium benzoate on physiological parameters support our findings. In a study conducted by Onyemaobi et al. (2012), different doses of sodium benzoate were applied to *A. cepa* L. bulbs and it was stated that the application inhibits root length and plant growth depending on the dose. In a study conducted by Rekha and Dharman (2011), it was stated that sodium benzoate application reduced germination in *A. cepa* L. In a study conducted by Moschetto et al. (2019), it was reported that sodium benzoate application inhibits plant growth and biomass increase in rice, and increasing application doses cause cell membrane structure disruption and photosynthetic pigment disorders. In a study conducted by Çavuşoğlu et al. (2017), it was reported that the germination percentage, weight gain, and radicle length decreased due to salinity in *A. cepa* L., showed improvement, and increased again with RJ application.

Genetic effects of benzoic acid and RJ were investigated with MI frequency, MN formation, CA frequency, and tail DNA formation. One of the two major endpoints of genotoxicity is the chromosomal abnormality test. The results of this test, which can be performed with many cells, have been successfully confirmed (Makoto 2007). The comet test is another important test that can detect genotoxic damage at the single-cell level. DNA chain breaks occur rapidly after genotoxic exposure, and the comet assay is sensitive in assessing this damage (Masood et al. 2012). The genetic effect of sodium benzoate application was realized as the decrease in MI rate, the formation of CAs and MN, increase in DNA tail percentage, and RJ application showed protective effects in all these parameters depending on the dose. Fragment and sticky chromosomes, which are the two most common and intensive chromosomal damage in research groups, can also illuminate the cause of other chromosomal damage occurrences.

Fragments can also cause secondary abnormalities by causing MN formation or inversion in the cells. Chromosome stickiness is associated with the toxic effects exhibited by chemicals and reflects the toxic effects of the generally irreversible type, most likely leading to cell death (Türkoğlu 2007; Fenech et al. 2016). The formation of the sticky chromosome is associated with the toxic effects of chemicals and can lead to cell death (Liu et al. 1992). The resulting sticky chromosome formation explains the reduction in MI. Sodium benzoate administration may have caused cell death and impairment in the mitotic cycle, leading to a decrease in mitotic activity. Chromosome breaks that occur in the form of fragments indicate the clastogenic potential of the chemical substance exposed (Saxena et al. 2005). MN formation occurring with sodium benzoate application can also be explained by clastogenic potential. Besides, the reason for the frequency of MN caused by sodium benzoate is thought to be the most common chromosome abnormality in the study, the adhesive chromosome. RJ application, on the other hand, showed a therapeutic effect depending on the dose, causing an increase in MI and a decrease in the percentage of MN, CAs, and DNA tail. RJ application reactivated the cell division cycle disrupted by sodium benzoate and caused an increase in cell division depending on the dose of administration. In the literature, there are studies conducted on the genetic effects of sodium benzoate and other food additives and support our findings. In the study conducted by Ali et al. (2020), the genotoxicity of sunset yellow and sodium benzoate food additives was investigated in cells, and as a result, it was reported that they cause chromosomal abnormalities in the form of chromatid breakage, fragmentation, ring chromosome and central fusion of chromosomes, and an increase in the frequency of nucleus tail formation and its frequency. In another study conducted by Aledwany et al. (2018), the genotoxicity of sodium benzoate and potassium nitrate, which are preservative food additives, was investigated with the *Drosophila* test model, and as a result, it was stated that both chemicals increased the

percentage of tail DNA and these chemicals were genotoxic. In a study conducted by Şahin et al. (2015), the mutagenicity of sodium benzoate was investigated on *Drosophila* and it was reported that sodium benzoate caused an increase in the DNA tail and tail moment depending on the application dose. Other studies have also reported that sodium benzoate causes MN formation and reduces MI, and this effect occurs depending on the application dose (Zengin et al. 2011; Pongsavee 2015; Kumar and Pandey 2015; Saatci et al. 2016; Lestari et al. 2017). Studies have also stated that RJ has a suppressing effect on genotoxicity. It has been reported that RJ reduces chromosomal abnormalities induced by valproic acid (Galaly et al. 2014); shows a dose-dependent preventive effect against chromosomal abnormalities, MN formation, and oxidative stress induced by cadmium (Çavuşoğlu et al. 2009); and reduces genotoxicity induced by doxorubicin (Jenkhetkan et al. 2018).

Lipid peroxidation and antioxidant enzyme activities increased due to sodium benzoate application in root tip tissues of *A. cepa* L. bulbs. This was determined by the increase in MDA, GR, SOD, and CAT levels. RJ application in combination with sodium benzoate caused significant decreases in MDA level and antioxidant enzyme levels depending on the application dose. That is, it showed dose-dependent therapeutic effects in both lipid peroxidation and antioxidant enzyme activity levels. In addition, hydrogen bonds and hydrophobic interactions between sodium benzoate and residues of GR, SOD, and CAT antioxidant enzymes were determined by molecular docking analysis. This has shown that sodium benzoate can affect the enzyme activities and functions by interacting with antioxidant enzyme residues, and explains the dramatic increases in GR, SOD, and CAT levels. Lipid peroxidation products such as MDA show their effects by changing basic molecules such as proteins and DNA bases (Madkour 2020), and it is known that free radicals can cause DNA damage in chromosomes (Aitken and Koppers 2011). There are defense mechanisms to protect cells against the harmful effects of free oxygen radicals. SOD, CAT, and GR are some of the enzymes of the antioxidant defense mechanism. Changes in these enzyme levels are markers of oxidative stress (Channarayappa and Biradar 2018). Sodium benzoate application caused lipid peroxidation and an increase in antioxidant enzyme levels. RJ applied in combination with sodium benzoate caused a dose-dependent improvement in all these parameters and reduced oxidative stress. Other studies have also reported that sodium benzoate administration caused oxidative stress. Sodium benzoate administration resulted in decreased glutathione level in the mouse brain tissue and increased MDA levels (Khoshnoud et al. 2018), caused oxidative stress in zebrafish (Gaur et al. 2018), and increased MDA level, and decreased SOD and CAT activities in human erythrocytes (Yetuk et al. 2014) have been stated.

In this study, in which the effects of RJ on all physiological, genetic, and biochemical parameters were evaluated

separately, it was seen that RJ exerted dose-dependent therapeutic effects on all parameters. It has been suggested that RJ shows protective effects by regulating the antioxidant system and reducing reactive oxygen species and that RJ is an activator of antioxidant enzymes (Cihan et al. 2013). Dose-dependent improvements in all parameters indicate that RJ is a potent and safe antioxidant affects oxidative stress–related toxicities (Najafi et al. 2014). Other studies have also confirmed that RJ exhibits antioxidant activity (Park et al. 2019; Gu et al. 2018). Different researches have also reported that RJ exerts a protective effect against toxicity induced by various chemical agents. It has been stated that RJ shows protective effects against physiological inhibition decrease, MI rate and chromosomal abnormalities in salinity stress (Çavuşoğlu et al. 2017), against oxidative stress induced by ofloxacin in rats (Manas and Najafi 2017), lipid peroxidation, and DNA damage induced by oxymetholone (Zahmatkesh et al. 2014).

It has been understood that the triggers of all these toxic effects caused by sodium benzoate are oxidative stress, the resulting oxidative stress does not disappear due to interactions of sodium benzoate with antioxidant enzyme residues, and this causes genetic damage. The decrease in MI can be explained by the fact that the cell cannot continue to divide and can exit the mitotic cycle due to genetic damage. The decrease in MI shows itself macroscopically with the decrease in rooting percentage root length and weight gain parameters. RJ application, on the other hand, reduces oxidative stress by acting as an antioxidant and accordingly shows dose-dependent healing effects on all parameters.

Conclusion

The goal of this research is to assess sodium benzoate toxicity and the possible therapeutic effects of royal jelly together for the first time. Sodium benzoate application had negative effects on all parameters, and the application of RJ triggered a partial inhibition of toxicity by showing dose-related therapeutic effects. Data from the study showed that sodium benzoate administration caused inhibition of physiological growth parameters, cytotoxic and genotoxic effects, and oxidative stress in *A. cepa* L. test material. It has also been found to interact with antioxidant enzymes. RJ administration showed dose-dependent therapeutic effects resulting in a partial improvement in all this toxicity.

The study showed that sodium benzoate impacts the division and growth of cells and affects DNA by inducing oxidative stress that negatively affects physiological development, and interacts with antioxidant enzymes aimed at reducing oxidative damage. RJ application reduced oxidative stress and DNA damage depending on the dose, and this showed itself with an improvement in physiological parameters. As a result, it has been revealed that oxidative stress, genetic damage, and

its interaction with the enzymes, caused by sodium benzoate, may cause toxic effects on those who use this food additive, and it is necessary to clarify the metabolic and cellular processes of sodium benzoate in humans. It was concluded that abandoning the use of this food additive if possible would be beneficial for the ecosystem and human health, and the use of RJ in daily nutrition would be effective in reducing the impact of toxic components exposed. Besides, it was concluded that it would be useful to determine the bioactive ingredients in RJ content and to investigate their usability in the pharmaceutical industry.

Author contribution AA carried out the experimental design, all experimental procedures, statistical analysis, and preparation of the article. The author read and approved the final manuscript.

Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The author declares no conflicts of interest.

References

Acar A (2021) Ameliorative effects of cape gooseberry (*Physalis peruviana* L.) against monosodium glutamate (MSG)–induced toxicity: genetic and biochemical approach. Environ Sci Pollut Res (2020). https://doi.org/10.1007/s11356-020-11800-1

Acar A, Çavuşoğlu K, Türkmen Z, Çavuşoğlu K, Yalçın E (2015) The investigation of genotoxic, physiological and anatomical effects of paraquat herbicide on *Allium cepa* L. Cytologia 80:343–351. https://doi.org/10.1508/cytologia.80.343

Acar A, Türkmen Z, Çavuşoğlu K, Yalçın E (2020) Investigation of benzyl benzoate toxicity with anatomical, physiological, cytogenetic and biochemical parameters in *in vivo*. Caryologia 73:21–32

Aitken RJ, Koppers AJ (2011) Apoptosis and DNA damage in human spermatozoa. Asian J Androl 13:36–42. https://doi.org/10.1038/aja.2010.68

Aledwany AZ, Basal WT, Al-Senosy NK, Issa AM (2018) Assessment of genotoxicity of potassium nitrate and sodium benzoate in *Drosophila melanogaster* using smart and comet assays. Egypt Acad J Biol Sci C 10:83–97. https://doi.org/10.21608/eajbsc.2018.22715

Ali MY, Hassan GM, Hassan AMS, Mohamed ZA, Ramadan MF (2020) *In vivo* genotoxicity assessment of sunset yellow and sodium benzoate in female rats. Drug Chem Toxicol 43:504–513. https://doi.org/10.1080/01480545.2018.1510416

Andrade MA, Riberio-Santos R, Nabavi SM, Sanches-Silva A (2020) Indirect Additives. In: Nabavi SM, Nabavi SF, Loizzo MR, Tundis R, Devi KP, Silva AS (eds) Food additives and human health. Bentham Science Publishers, Sharjah, pp 246–248

Atik M, Karagüzel O, Ersoy S (2007) Effect of temperature on germination characteristics of *Dalbergia sissoo* seeds. Mediterr Agric Sci 20:203–210 (in Turkish)

Babatunde BB, Vincent-Akpu IF, Aiwerioghene ANO (2016) Cytogenotoxicity screening of untreated hospital wastewaters using the *Allium cepa* test. J Appl Sci Environ Manag 20:724–732. https://doi.org/10.4314/jasem.v20i3.27

Banci L, Benedetto M, Bertini I, Del Conte R, Piccioli M, Viezzoli MS (1998) Solution structure of reduced monomeric Q133M2 copper, zinc superoxide dismutase (SOD). Why is SOD a dimeric enzyme? Biochemistry 37:11780–11791. https://doi.org/10.1021/bi9803473

Barnutiu LI, Marghitaş LA, Dezmirean DS, Mihai CM, Bobiş O (2011) Chemical composition and antimicrobial activity of royal jelly-review. Sci Pap Anim Sci Biotechnol 44:67–72

Bearth A, Cousin ME, Siegrist M (2014) The consumer's perception of artificial food additives: influences on acceptance, risk and benefit perceptions. Food Qual Prefer 38:14–23. https://doi.org/10.1016/j.foodqual.2014.05.008

Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44:276–287. https://doi.org/10.1016/0003-2697(71)90370-8

Beers RF, Sizer IW (1952) Colorimetric method for estimation of catalase. J Biol Chem 195:133–139

Bhat SA, Cui G, Li F, Vig AP (2019) Biomonitoring of genotoxicity of industrial wastes using plant bioassays. Bioresour Technol Rep 6:207–216. https://doi.org/10.1016/j.biteb.2019.03.005

Bonciu E, Firbas P, Fontanetti CS, Wusheng J, Karaismailoğlu MC, Liu D, Menicucci F, Pesnya DS, Popescu A, Romanovsky AV, Schiff S, Joanna S, de Souze CP, Stivastava A, Sultan A, Papini A (2018) An evaluation for the standardization of the Allium cepa test as cytotoxicity and genotoxicity assay. Caryologia 71:191–209. https://doi.org/10.1080/00087114.2018.1503496

Burdock GA (1997) Encyclopedia of food and color additives, vol 3. CRC Press, Florida, pp 2527–2528

Bystrzejewska-Piotrowska G, Urban PL (2004) Accumulation and translocation of cesium-137 in onion plants (*Allium cepa*). Environ Exp Bot 51:3–7. https://doi.org/10.1016/S0098-8472(03)00039-X

Calvo-Flores FG, Isac-García J, Dobado JA (2018) Emerging pollutants: origin, structure, and properties. Wiley, Weinheim, pp 238–250

Carlberg I, Mannervik B (1975) Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J Biol Chem 250:5475–5480

Chakraborty R, Mukherjee AK, Mukherjee A (2009) Evaluation of genotoxicity of coal fly ash in *Allium cepa* root cells by combining comet assay with the *Allium* test. Environ Monit Assess 153:351–357. https://doi.org/10.1007/s10661-008-0361-z

Channarayappa C, Biradar DP (2018) Abiotic Stress. In: Channarayappa C, Biradar DP (eds) Soil basics, management and rhizosphere engineering for sustainable agriculture. CRC Press, London, pp 609–647. https://doi.org/10.1201/9781351044271

Cihan YB, Ozturk A, Gokalp SS (2013) Protective role of royal jelly against radiation-induced oxidative stress in rats. Int J Hematol Oncol 29:79–87. https://doi.org/10.4999/uhod.11016

Clipley JR (2020) Sodium benzoat and benzoic acid. In: Davidson PM, Taylor TM, David JR (eds) Antimicrobials in food. CRC Press, London, pp 41–88

Croom E (2012) Metabolism of xenobiotics of human environments. In: Hongson E (ed) Progress in molecular biology and translational science. Academic Press, California, pp 31–88

Çavuşoğlu K, Yapar K, Yalçin E (2009) Royal jelly (honey bee) is a potential antioxidant against cadmium-induced genotoxicity and oxidative stress in albino mice. J Med Food 12:1286-1292. https://doi.org/10.1089/jmf.2008.0203

Çavuşoğlu D, Tabur S, Çavuşoğlu K (2017) Physiological and cytogenetical effects of royal jelly (honey bee) in *Allium cepa* L. seeds

exposed to salinity. Cytologia 82:115–121. https://doi.org/10.1508/cytologia.82.115

Çıldır DS, Liman R (2020) Cytogenetic and genotoxic assessment in *Allium cepa* exposed to imazalil fungicide. Environ Sci Pollut Res 27:20335–20343. https://doi.org/10.1007/s11356-020-08553-2

EFSA (2016) EFSA Panel on Food Additives and Nutrient Sources (ANS) Scientific opinion on the re-evaluation of benzoic acid (E 210), sodium benzoate (E 211), potassium benzoate (E 212) and calcium benzoate (E 213) as food additives. EFSA J 14:4433. https://doi.org/10.2903/j.efsa.2016.4433

Esimbekova EN, Asanova AA, Deeva AA, Kratasyuk VA (2017) Inhibition effect of food preservatives on endoproteinases. Food Chem 235:294–297. https://doi.org/10.1016/j.foodchem.2017.05.059

Fenech M, Chang WP, Kirsch-Volders M, Holland N, Bonassi S, Zeiger E (2003) HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. Mutat Res 534:65–75. https://doi.org/10.1016/S1383-5718(02)00249-8

Fenech M, Knasmueller S, Bolognesi C, Bonassi S, Holland N, Migliore L, Palitti F, Natarajan AT, Kirsch-Volders M (2016) Molecular mechanisms by which *in vivo* exposure to exogenous chemical genotoxic agents can lead to micronucleus formation in lymphocytes *in vivo* and *ex vivo* in humans. Mutat Res Rev Mutat Res 770:12–25. https://doi.org/10.1016/j.mrrev.2016.04.008

Fioresi VS, de Cássia Ribeiro Vieira B, de Campos JMS, da Silva ST (2020) Cytogenotoxic activity of the pesticides imidacloprid and iprodione on *Allium cepa* root meristem. Environ Sci Pollut Res 27:28066–28076. https://doi.org/10.1007/s11356-020-09201-5

Galaly SR, Abdella EM, Mohammed HM (2014) Effects of royal jelly on genotoxicity and nephrotoxicity induced by valproic acid in albino mice. Beni-Suef Univ J Basic Appl Sci 3:1–15. https://doi.org/10.1016/j.bjbas.2014.02.001

Gaur H, Purushothaman S, Pullaguri N, Bhargava Y, Bhargava A (2018) Sodium benzoate induced developmental defects, oxidative stress and anxiety-like behaviour in zebrafish larva. Biochem Biophys Res Commun 502:364–369. https://doi.org/10.1016/j.bbrc.2018.05.171

Grant WF (1982) Chromosome aberration assays in Allium: a report of the US Environmental Protection Agency gene-tox program. Mutat Res 99:273–291. https://doi.org/10.1016/0165-1110(82)90046-X

Gu H, Song IB, Han HJ, Lee NY, Cha JY, Son YK, Kwon J (2018) Antioxidant activity of royal jelly hydrolysates obtained by enzymatic treatment. Korean J Food Sci Anim Resour 38:135–142. https://doi.org/10.5851/kosfa.2018.38.1.135

Guex N, Peitsch MC (2005) SWISS-MODEL and the Swiss-Pdb Viewer: an environment for comparative protein modeling. Electrophoresis 18:2714–2723. https://doi.org/10.1002/elps.1150181505

Isidorov VA, Czyżewska U, Isidorova AG, Bakier S (2009) Gas chromatographic and mass spectrometric characterization of the organic acids extracted from some preparations containing lyophilized royal jelly. J Chromatogr B 877:3776–3780. https://doi.org/10.1016/j.jchromb.2009.09.016

Jenkhetkan W, Thitiorul S, Jansom C, Ratanavalachai T (2018) Genoprotective effects of thai royal jelly against doxorubicin in human lymphocytes in vitro. Nat Prod Commun 13:79–84. https://doi.org/10.1177/1934578X1801300124

Khoshnoud MJ, Siavashpour A, Bakhshizadeh M, Rashedinia M (2018) Effects of sodium benzoate, a commonly used food preservative, on learning, memory, and oxidative stress in brain of mice. J Biochem Mol Toxicol 32:1–7. https://doi.org/10.1002/jbt.22022

Końca K, Lankoff A, Banasik A, Lisowska H, Kuszewski T, Góźdź S, Koza Z, Wojcik A (2003) A cross-platform public domain PC image-analysis program for the comet assay. Mutat Res 534:15–20. https://doi.org/10.1016/s1383-5718(02)00251-6

Kumar G, Pandey A (2015) Genotoxic and mito-depressive effects of food preservatives on root meristems of barley (*Hordeum vulgare* L.). Chromosome Bot 10:51–60. https://doi.org/10.3199/iscb.10.51

Lennerz BS, Vafai SB, Delaney NF, Clish AB, Deik AA, Pierce KA, Ludwig DS, Mootha VK (2015) Effect of sodium benzoate, a widely used food preservative, on glucose homeostasis and metabolic profiles in humans. Mol Genet Metab 114:73–79. https://doi.org/10.1016/j.ymgme.2014.11.010

Lestari B, Novitasari D, Putri H, Haryanti S, Sasmito E, Meiyanto E (2017) Evaluation of the genotoxicity of three food additives using CHO-K1 Cells under in vitro micronucleus flow cytometry assay. Indones J Canc Chemoprev 8:74–80

Liu D, Jiang W, Li M (1992) Effects of trivalent and hexavalent chromium on root growth and cell division of *Allium cepa*. Hereditas 117:23–29. https://doi.org/10.1111/j.1601-5223.1992.tb00003.x

Madkour LH (2020) Reactive oxygen species (ROS), nanoparticles, and endoplasmic reticulum (er) stress-induced cell death mechanisms. Academic Press, California, pp 186–187

Makoto H (2007) *In vivo* rodent micronucleus assay. In: Obe G (ed) Chromosomal alterations: methods, results and importance in human health. Springer-Verlag, Heidelberg, pp 257–270

Manas GE, Najafi G (2017) Protective effects of royal jelly on the histomorphologic, oxidative stress and sperm parameters in ofloxacin treated rat. Comp Clin Pathol 26:1111–1115. https://doi.org/10.1007/s00580-017-2494-3

Masood F, Anjum R, Ahmad M, Malik A (2012) Methods for genotoxicity testing of environmental pollutants. In: Malik A, Grohmmann E (eds) Environmental protection strategies for sustainable development. Springer, Dordrecht, pp 229–260. https://doi.org/10.1007/978-94-007-1591-2_7

Moschetto FA, Lopes MF, Silva BP, Neto MCL (2019) Sodium benzoate inhibits germination, establishment and development of rice plants. Theor Exp Plant Physiol 31:377–385. https://doi.org/10.1007/s40626-019-00151-z

Najafi G, Nejati V, Shalizar Jalali A, Zahmatkesh E (2014) Protective role of royal jelly in oxymetholone-induced oxidative injury in mouse testis. Iran J Toxicol 8:1073–1080

Nakajima Y, Tsuruma K, Shimazawa M, Mishima S, Hara H (2009) Comparison of bee products based on assays of antioxidant capacities. BMC Complement Altern Med 9:4. https://doi.org/10.1186/1472-6882-9-4

O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR (2011) Open Babel: an open chemical toolbox. J Cheminform 3:33. https://doi.org/10.1186/1758-2946-3-33

Oladokun EI, Sogbanmu TO, Anikwe JC (2020) Sublethal concentrations of dichlorvos and paraquat induce genotoxic and histological effects in the *Clarias gariepinus*. Environ Anal Health Toxicol 35:2020013. https://doi.org/10.5620/eaht.2020013

Onyemaobi OI, Williams GO, Adekoya KO (2012) Cytogenetic effects of two food preservatives, sodium metabisulphate and sodium benzoate on root tips of *Allium cepa* Linn. IFE J Sci 14:155–165

Park MJ, Kim BY, Park HG, Deng Y, Yoon HJ, Choi YS, Lee KS, Jin BR (2019) Major royal jelly protein 2 acts as an antimicrobial agent and antioxidant in royal jelly. J Asia Pac Entomol 22:684–689. https://doi.org/10.1016/j.aspen.2019.05.003

Pongsavee M (2015) Effect of sodium benzoate preservative on micronucleus induction, chromosome break, and Ala40Thr superoxide dismutase gene mutation in lymphocytes. Biomed Res Int 2015:1–5. https://doi.org/10.1155/2015/103512

Praveen A, Gupta M (2018) Nitric oxide confronts arsenic stimulated oxidative stress and root architecture through distinct gene expression of auxin transporters, nutrient related genes and modulates biochemical responses in *Oryza sativa* L. Environ Pollut 240:950–962. https://doi.org/10.1016/j.envpol.2018.04.096

Purich DL (2010) Enzyme kinetics: catalysis and control: a reference of theory and best-practice methods. Elsevier, California, pp 60–61

Qiu W, Chen X, Tian Y, Wu D, Du M, Wang S (2020) Protection against oxidative stress and anti-aging effect in *Drosophila* of royal jelly-collagen peptide. Food Chem Toxicol 135:110881. https://doi.org/10.1016/j.fct.2019.110881

Ramadan MF, Al-Ghamdi A (2012) Bioactive compounds and health-promoting properties of royal jelly: a review. J Funct Foods 4:39–52. https://doi.org/10.1016/j.jff.2011.12.007

Rekha K, Dharman AK (2011) Mitotic aberrations induced by sodium benzoate: a food additive in *Allium cepa* L. Plant Arch 11:945–947

Rodrigues GZP, Machado AB, Finkler M, Berleze DB, Gehlen G (2020) Environmental assessment of luiz Rau Stream (Brazil) utilizing *Allium cepa* test. Ci e Nat 42:76. https://doi.org/10.5902/2179460X41818

Saad B, Bari MF, Saleh MI, Ahmad K, Talib MKM (2005) Simultaneous determination of preservatives (benzoic acid, sorbic acid, methylparaben and propylparaben) in foodstuffs using high-performance liquid chromatography. J Chromatogr A 1073:393–397. https://doi.org/10.1016/j.chroma.2004.10.105

Saatci C, Erdem Y, Bayramov R, Akalın H, Tascioglu N, Ozkul Y (2016) Effect of sodium benzoate on DNA breakage, micronucleus formation and mitotic index in peripheral blood of pregnant rats and their newborns. Biotechnol Biotechnol Equip 30:1179–1183. https://doi.org/10.1080/13102818.2016.1224979

Saxena PN, Chauhan LKS, Gupta SK (2005) Cytogenetic effects of commercial formulation of cypermethrin in root meristem cells of *Allium sativum*: spectroscopic basis of chromosome damage. Toxicology 216:244–252. https://doi.org/10.1016/j.tox.2005.08.008

Shahmohammadi M, Javadi M, Nassiri-Asl M (2016) An overview on the effects of sodium benzoate as a preservative in food products. Biotechnol Health Sci 3:7–11. https://doi.org/10.17795/bhs-35084

Staykova TA, Ivanova EN, Velcheva IG (2005) Cytogenetic effect of heavy metal and cyanide in contamined waters from the region of Southwest Bulgaria. J Mol Cell Biol 4:41–46

Stocker A (2003) Isolation and characterisation of substances from royal jelly. Technische Universität München, Munich, pp 1–202

Sugiyama T, Takahashi K, Mori H (2012) Royal jelly acid, 10-hydroxy-trans-2-decenoic acid, as a modulator of the innate immune responses. Endocr Metab Immune Disord Drug Targets 12:368–376. https://doi.org/10.2174/187153012803832530

Şahin N, Pirinc B, Türkoğlu Ş (2015) In vivo genotoxicity assessment of some food preservatives in *Drosophila melanogaster* with the comet assay. Fresenius Environ Bull 24:2138–2145

Tohamy HG, El-Karim DRG, El-Sayed YS (2019) Attenuation potentials of royal jelly against hydroxyurea-induced infertility through inhibiting oxidation and release of pro-inflammatory cytokines in male rats. Environ Sci Pollut Res 26:21524–21534. https://doi.org/10.1007/s11356-019-05521-3

Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 31:455–461. https://doi.org/10.1002/jcc.21334

Turkez H, Aydın E (2013) Anti-genotoxic role of eicosapentaenoic acid against imazalil-induced DNA damage in vitro. Toxicol Ind Health 29:584–590. https://doi.org/10.1177/0748233711433943

Türkmen Z, Çavuşoğlu K, Çavuşoğlu K, Yapar K, Yalçın E (2009) Protective role of royal jelly (honeybee) on genotoxicity and lipid peroxidation, induced by petroleum wastewater, in *Allium cepa* L. root tips. Environ Technol 30:1205–1214. https://doi.org/10.1080/09593330903179757

Türkoğlu Ş (2007) Genotoxicity of five food preservatives tested on root tips *of Allium cepa* L. Mutat Res Genet Toxicol Environ Mutagen 626:4–14. https://doi.org/10.1016/j.mrgentox.2006.07.006

Ünyayar S, Celik A, Çekiç FÖ, Gözel A (2006) Cadmium-induced genotoxicity, cytotoxicity and lipid peroxidation in *Allium sativum* and *Vicia faba*. Mutagenesis 21:77–81. https://doi.org/10.1093/mutage/gel001

Vicentini VEP, Camparoto ML, Teixeira RO, Mantovani MS (2001) *Averrhoa carambola* L., *Syzygium cumini* (L.) Skeels and *Cissus sicyoides* L.: medicinal herbal tea effects on vegetal and test systems. Acta Sci Biol Sci 23:593–598

Waykar BB, Alqadhi YA (2020) Protective role of royal jelly and honey against cisplatin induced sperm function parameters in male rats. Indian J Public Health Res Dev 11:153–158

Xue X, Wu L, Wang K (2017) Chemical composition of royal jelly. In: Alvarez-Suarez JM (ed) Bee products-chemical and biological properties. Springer, Cham, pp 181–190

Yadav A, Kumar A, Das M, Tripathi A (2016) Sodium benzoate, a food preservative, affects the functional and activation status of splenocytes at non cytotoxic dose. Food Chem Toxicol 88:40–47. https://doi.org/10.1016/j.fct.2015.12.016

Yetuk G, Pandir D, Bas H (2014) Protective role of catechin and quercetin in sodium benzoate-induced lipid peroxidation and the antioxidant system in human erythrocytes in vitro. Sci World J 2014:1–6. https://doi.org/10.1155/2014/874824

Yonekura K, Maki-Yonekura S (2016) Refinement of cryo-EM structures using scattering factors of charged atoms. J Appl Crystallogr 49:1517–1523. https://doi.org/10.1107/S1600576716011274

Yu J, Zhou CZ (2007) Crystal structure of glutathione reductase Glr1 from the yeast Saccharomyces cerevisiae. Proteins 68:972–979. https://doi.org/10.1002/prot.21354

Zahmatkesh E, Najafi G, Nejati V, Heidari R (2014) Protective effect of royal jelly on the sperm parameters and testosterone level and lipid peroxidation in adult mice treated with oxymetholone. Avicenna J Phytomed 4:43–52

Zengin N, Yüzbaşıoğlu D, Ünal F, Yılmaz S, Aksoy H (2011) The evaluation of the genotoxicity of two food preservatives: sodium benzoate and potassium benzoate. Food Chem Toxicol 49:763–769. https://doi.org/10.1016/j.fct.2010.11.040