



Protective role of green tea against paraquat toxicity in *Allium cepa* L.: physiological, cytogenetic, biochemical, and anatomical assessment

Ferhat Yirmibeş¹ · Emine Yalçın² · Kültiğın Çavuşoğlu²

Received: 13 September 2021 / Accepted: 27 October 2021 / Published online: 24 November 2021
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract

In this study, the toxic effects of paraquat, one of the most commercially sold herbicides in the world, and the protective role of green tea leaf extract (GTLE) against these effects were investigated. *Allium cepa* L. bulbs ($n=16$) were used as test material. One hundred milligrams per liter dose of paraquat and 190 and 380 mg/L doses of GTLE were preferred. Paraquat toxicity was investigated with the help of physiological (percent germination, root length, and weight gain), cytogenetic (mitotic index = MI, micronucleus = MN, and chromosomal damages = CAs), biochemical (superoxide dismutase = SOD, catalase = CAT, malondialdehyde = MDA), and anatomical (meristematic cell damages) parameters. *A. cepa* bulbs were divided into 6 groups as 1 control and 5 applications. The control group was germinated with tap water, and the application groups were germinated with paraquat and two different doses of GTLE. Germination was carried out at room temperature for 72 h. At the end of the period, *A. cepa* bulbs were prepared for physiological, cytogenetic, biochemical, and anatomical analyzes using routine preparation techniques. As a result, paraquat application caused a decrease in physiological parameters and an increase in cytogenetic (except MI) and biochemical parameters. Compared to the control (group I), the germination percentage decreased by 38%, root length 12.5 times, and weight gain 5 times decreased in group IV treated with paraquat. MDA level increased 2.58 times, SOD activity 2.48 times, and CAT activity 4.51 times increased. Paraquat application caused a decrease in the percentage of MI and an increase in the number of MN and CAs. Paraquat application caused CAs in the form of fragment, sticky chromosome, unequal distribution of chromatin, bridge, nucleus with vacuoles, nucleus bud, and reverse polarization. In the meristematic cells of the root tips applied paraquat, unclearly vascular tissue, flattened cell nucleus, epidermis, and cortex cell deformation were observed. The application of GTLE together with paraquat caused an increase in the physiological parameter values and a decrease in the cytogenetic (except MI) and biochemical parameter values. An improvement in the severity of damages induced by paraquat was also observed in root tip meristematic cells. It was determined that the improvements observed in all these parameters were related to the dose of GTLE applied. The 380 mg/L dose of GTLE provided more protection than the 190 mg/L dose. Compared to group IV in which paraquat was applied, the germination percentage increased by 21%, root length 5.83 times, and weight gain 2.92 times increased in group VI administered 380 mg/L dose of GTLE. In addition, MDA level decreased 1.78 times, SOD activity 1.59 times and CAT activity 1.65 times. In conclusion, paraquat administration at a dose of 100 mg/L caused physiological, cytogenetic, biochemical, and anatomical toxicity in *A. cepa* bulbs. GTLE application, on the other hand, resulted in improvements in the severity of this toxicity induced by paraquat, depending on the dose. Therefore, GTLE can be used as an effective nutritional supplement to reduce or prevent the toxicity caused by environmental agents such as pesticides.

Keywords *Allium cepa* L · Anatomy · Antioxidant enzymes · Chromosomal damage · Lipid peroxidation · Micronucleus · Paraquat · Green tea extract

Responsible Editor: Gangrong Shi

✉ Kültiğın Çavuşoğlu
kultigin.cavusoglu@giresun.edu.tr

¹ Institute of Science, Giresun University, Giresun, Turkey

² Faculty of Science and Art, Department of Biology, Giresun University, Giresun, Turkey

Introduction

A pesticide is a chemical substance used to destroy, to prevent their reproduction or to control them, vectors, invasive plant, or animal species that cause disease in humans, domestic animals, and agricultural products. In other words,

pesticides are toxic chemicals that are released into the environment to combat pests (Zacharia 2011). However, most of the pesticides applied to combat pests spread to the environment and cause various health problems in non-target organisms together with environmental pollution. People are especially exposed to pesticides, during pesticide application in agricultural areas, consuming pesticide-contaminated food and water, or loading and transporting pesticide products. Pesticides enter the structure of organisms via water, soil, roots, leaves, skin, respiration, and nutrition. They cause acute and chronic toxicity in living things, promoting reversible or irreversible damage (Tosun et al. 2001; Hashmi and Khan 2011). Pesticides are classified according to a wide variety of criteria. The most widely used of these is the classification made according to the harmful organism on which pesticides act and the function of the pesticide. Accordingly, it is possible to classify pesticides as acaricides, algicides, insecticides, avicides, fungicides, and herbicides (Akashe et al. 2018).

Herbicides are chemical or biological agents used to specifically kill unwanted plants or inhibit their growth. Herbicides can be a naturally derived or synthetically produced substance. Each herbicide contains an active ingredient. Herbicides containing active ingredients act by disrupting various physical and biochemical processes of plants. For example, some herbicides have a lethal effect by disrupting the cellular membranes of plants and leaking the cell contents out. Others affect specific processes such as respiration, photosynthesis, or the production of aliphatic amino acids. In general, enzymes are key molecules in plant cells that perform functions necessary for normal plant growth. Therefore, herbicides abolish the activities of plant enzymes by binding to them from a target site. Thus, they cause the disruption of a certain process and the death of the plant (Tu et al. 2001).

Paraquat is a nitrogenous herbicide expressed as 1,1'-dimethyl-4,4'-bipyridium dichloride. It is widely used around the world due to its high productivity and non-selective property, especially in the control of broadleaf weeds. It is the second best-selling herbicide in the world with its availability rate. It is highly toxic to different organisms. It causes acute respiratory distress syndrome (ARDS) in humans. It is also reported that intense paraquat toxicity causes multi-organ failure in the kidneys, liver, and lungs and increases the risk of developing Parkinson's disease. It has also been reported that paraquat plays a role in the development of leukemia, lymphoma, and brain and skin cancers. Death is due to respiratory failure due to advanced pulmonary fibrosis. Although the mechanism by which paraquat produces toxicity has not been fully elucidated, the toxicity is thought to result from the production of reactive oxygen species (ROS) via the redox cycle. Because these radicals are highly reactive, they are thought to promote oxidative

stress induced damages to cellular organelles, chlorophyll, protein, fatty acids, nucleic acids, and lipids. This situation, especially in plants, disrupts the integrity of the cell membrane, causing rapid wilting and rusting of the leaves, and eventually the death of the plant. Today, although the EU bans the use of paraquat, it is still used extensively as a pesticide in many countries (Acar et al. 2015; Reddy et al. 2019).

Tea is a type of beverage obtained by boiling the leaves, flowers, and seeds of the *Camellia sinensis* L. plant. Tea is the second most consumed beverage in the world after water. Since the beneficial effects of tea consumption in human diseases show promising results, it has been used as an antioxidant beverage in alternative medicine for centuries in India and China. *C. sinensis* is a perennial and evergreen plant that grows naturally in the tropical and subtropical forests of the world. Although its homeland is South and South East Asia, it is also widely cultivated in some countries of Africa and the Middle East. Green tea (GT) is obtained by collecting the leaves of *C. sinensis*, slightly steaming and drying. It has many antioxidant molecule groups such as flavonoids, flavonols, polyphenols, theaflavins, and tannins. Catechins are highly potent flavonoids found in tea. The most abundant are epigallocatechin (EGC), epicatechin (EC), epicatechin gallate (EKG), and epigallocatechin gallate (EGCG). The antioxidant activity of this catechin is approximately 25–100 times stronger than vitamins E and C. Polyphenols and catechins found in tea prevent the formation of free radicals and play a role in scavenging of ROS. In this case, it makes GT have anti-inflammatory, antioxidant, photoprotective, and chemopreventive properties. Some studies carried out in recent years have revealed that GT extracts are effective in suppressing environmentally induced breast cancers, autoimmune diseases, inflammatory responses in coronary vessels, and protect genetic material. In addition, GT has shown positive effects in skin diseases and carcinogenesis. All these results have caused becoming a popular trend of GT consumption in western cultures in today (Gupta et al. 2009; OyetakinWhite et al. 2012; Mohabbulla Mohib et al. 2016).

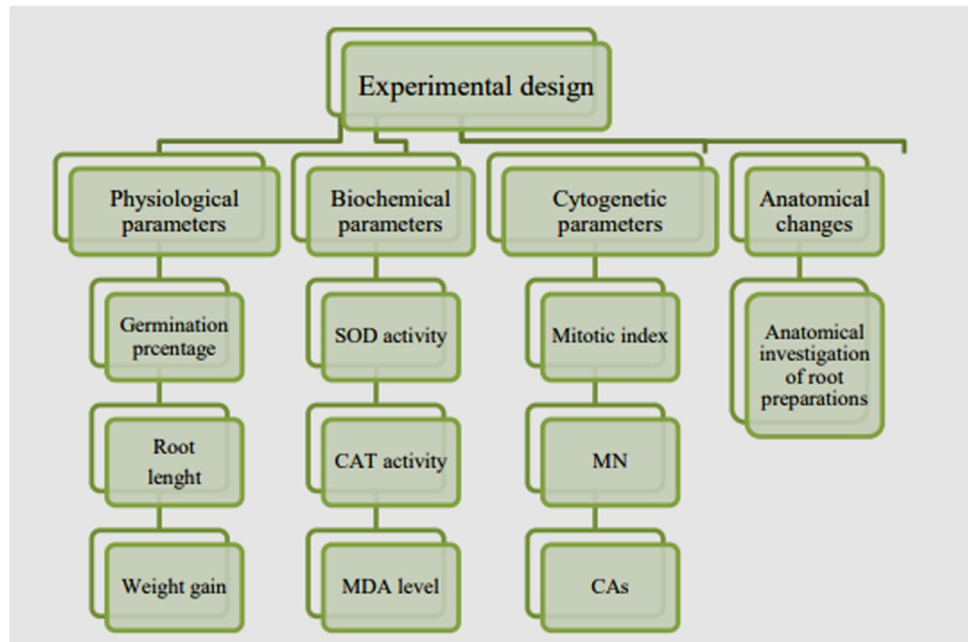
The aim of this study is to investigate the toxic effects of paraquat which one of the herbicides widely used worldwide especially in the fight against weeds, and the protective role of GTLE against these effects, with the help of cytogenetic, physiological, anatomical, and biochemical parameters in *Allium cepa* test material.

Materials and methods

Experimental design

In this study, the protective effects of GTLE against paraquat toxicity were investigated. Paraquat toxicity and GTLE

Diagram 1 Experimental design of study



protection were investigated using different parameters. All parameters used in the study are given in Diagram 1.

Test material and experimental groups

Commercially available *A. cepa* bulbs were used as test material and GTLE (60 capsules × 380 mg) from SepeNatural Company as protective biological product. Bulbs are divided into 6 groups as follows.

- Group I: Control.
- Group II: 190 mg/L GTLE.
- Group III: 380 mg/L GTLE.
- Group IV: 100 mg/L paraquat.
- Group V: 100 mg/L paraquat + 190 mg/L GTLE.
- Group V: 100 mg/L paraquat + 380 mg/L GTLE.

Bulbs were germinated at room temperature for 72 h in sterile glass beakers (85 × 100 mL). During germination, tap water was applied to the bulbs of the control group and 100 mg/L of paraquat and two different doses of GTLE (190 and 380 mg/L) to the bulbs of the application group. During the germination period, all groups were checked daily, and the missing solutions were added. At the end of the period, the bulbs were washed with distilled water and prepared for experimental analysis with the help of routine preparation procedures (Wei 2004). The 100 mg/L dose of Paraquat was determined based on previous similar studies (Özen et al. 2011; Acar et al. 2015). GTLE doses were preferred by nutritionists based on the recommended dose amounts to be taken in the daily diet.

Physiological parameter analyzes

The effects of paraquat and GTLE treatments on physiological parameters were determined as follows.

- Effects on root length were determined by measuring radicle (embryonic root zone — the structure that forms roots in the mature plant) lengths with a millimetric ruler (mm).
- The effects on the weight were determined by weighing of the bulb weights with the help of precision scales before and after the experiment.
- The effects on germination were calculated using the formula in (Eq. 1) (Atik et al. 2007).

$$\text{Germination}(\%) = (\text{number of germinated seeds} / \text{total number of seeds}) \times 100 \quad (1)$$

Cytogenetic analyzes

In order to determine the cytogenetic parameters (CAs, MN, and MI), root slides were prepared according to Staykova et al. (2005). Detection and counting of CAs and presence of MN were made at × 500 magnification (Irmeco IM-450 TI) by two different observers and photographed. MN determination was made according to the criteria suggested by Fenech et al. (2003). MI was calculated as a percentage using Eq. (2).

$$\text{MI}(\%) = (\text{Dividing cell number} / \text{Total cell number}) \times 100 \quad (2)$$

Table 1 Effects of paraquat and GTLE on selected physiological parameters

Groups	Germination percent-age	Root length (cm)	Weight gain (g)
I	99	7.50 ± 1.85 ^a	+ 7.50 ^a (6.12 ± 0.65–13.62 ± 1.85)
II	98	7.40 ± 1.88 ^a	+ 7.62 ^a (6.32 ± 0.68–13.94 ± 1.90)
III	100	7.60 ± 1.83 ^a	+ 7.84 ^a (6.25 ± 0.66–14.09 ± 1.95)
IV	62	0.60 ± 0.77 ^d	+ 1.50 ^d (6.30 ± 0.67–7.80 ± 0.84)
V	71	1.40 ± 1.23 ^c	+ 2.66 ^c (6.15 ± 0.64–8.81 ± 0.92)
VI	83	3.50 ± 1.58 ^b	+ 4.38 ^b (6.20 ± 0.65–10.58 ± 1.16)

*Group I: control, group II: 190 mg/L GTLE, group III: 380 mg/L GTLE, group IV: 100 mg/L paraquat, group V: 100 mg/L paraquat + 190 mg/L GTLE, group VI: 100 mg/L paraquat + 380 mg/L GTLE. Data were given as mean ± SD. Different letters^(a–d) in the same column are statistically important at $p < 0.05$.

Biochemical analyzes

In biochemical analyzes, MDA level, SOD, and CAT activities were measured in root tip. MDA levels were measured by a standard protocol suggested by Ünyayar et al. (2006) and presented as $\mu\text{M g}^{-1}$ FW. Before SOD and CAT activity analysis extraction procedure was applied. Enzyme extraction was carried out at +4 °C. 0.5 g of root tips were washed with distilled water, homogenized in 5 mL of monosodium phosphate buffer. Homogenate was centrifuged at 10,500 g for 20 min, and the supernatant was used in biochemical analysis (Zou et al. 2012). SOD activity was measured in accordance with the method suggested by Beauchamp and Fridovich (1971) with a light induction analysis and the SOD activity shown as U mg^{-1} FW. CAT activity was measured in accordance with the method suggested by Beers and Sizer (1952) and shown as $\text{OD}_{240} \text{ nm min g}^{-1}$ (Zou et al. 2012).

Observation of anatomical damages

Root tips cut in 1 cm length were cleaned in distilled water and placed between foam materials. In the next step, cross-sections of samples were taken with the help of a heat-sterilized razor blade. The sections placed on the slide were stained with 5% methylene blue for 2 min, covered with a coverslip with the help of entellan, and the preparation process was completed. Detection of root tip meristematic cell damages were investigated with a research microscope at $\times 500$ magnification by two different observers and photographed (Çavuşoğlu et al. 2020).

Recovery effect of GTLE

Protective effect of GTLE (recovery effect = RE) against paraquat toxicity was calculated by using Eq. (5). In determining RE, data belonging to group VI, where GTLE showed the highest recovery, and data from group IV, in which paraquat was administered alone and control groups data were used.

RE values for all tested parameters were calculated separately (Gündüz et al. 2021).

$$RE(\%) = \left[\frac{(D_1 - D_2)}{(D_3 - D_2)} \right] \times 100 \quad (3)$$

D_1 is the data of paraquat + GTLE-treated group, D_2 is the data of paraquat-treated group, D_3 is the data of control.

Statistical analyzes

Experimental data were evaluated with the help of SPSS Statistics 22 (IBM SPSS, Turkey) package program. Data are shown as mean ± standard deviation (SD). The statistical significance between the means was determined using the “One-way ANOVA”, which are expressed as one-way analysis of variance and “Duncan” test. The obtained value was considered statistically significant when $p < 0.05$.

Result and discussion

Physiological effects

The effects of paraquat and GTLE application on selected physiological parameters in *A. cepa* are shown in Table 1. The highest weight gain, germination percentage, and root length were observed in the control group and in groups II and III, which only applied GTLE. Paraquat exposure, on the other hand, caused statistically significant ($p < 0.05$) decreases in all selected physiological parameters. Compared to the control group, germination percentage decreased by 38%, root length by 12.5 times and weight gain by 5 times in group IV exposed to paraquat. These decreases observed in physiological parameter values can be associated with the toxicity caused by paraquat directly in *A. cepa* tissues. It has been reported in the literature that paraquat is directly taken up by epidermal and cortical cells through a protein-mediated system that functions to transport polyamines in plant roots. In this way, the paraquat can move freely through the xylem and be transported to the roots, stems,

Table 2 Protective role of GTLE against paraquat-induced CAs

Damages	Control	190 mg/L GTLE	380 mg/L GTLE	100 mg/L Paraquat	100 mg/L Paraquat + 190 mg/L GTLE	100 mg/L Paraquat + 380 mg/L GTLE
DCN	894.00 ± 49.52 ^a	912.00 ± 50.75 ^a	905.00 ± 48.87 ^a	536.00 ± 31.90 ^d	613.00 ± 38.66 ^c	702.00 ± 45.48 ^b
MI (%)	8.94	9.12	9.05	5.36	6.13	7.02
MN	0.16 ± 0.24 ^d	0.12 ± 0.18 ^d	ND	56.00 ± 6.78 ^a	42.80 ± 5.53 ^b	29.50 ± 4.84 ^c
FRG	ND	ND	ND	70.40 ± 8.56 ^a	58.30 ± 7.14 ^b	41.70 ± 5.82 ^c
SC	0.16 ± 0.28 ^d	0.13 ± 0.20 ^d	0.10 ± 0.16 ^d	50.90 ± 6.88 ^a	41.50 ± 5.13 ^b	30.60 ± 4.72 ^c
UDC	ND	ND	ND	38.10 ± 5.24 ^a	26.20 ± 4.16 ^b	19.40 ± 3.56 ^c
B	ND	ND	ND	35.40 ± 4.68 ^a	25.90 ± 4.12 ^b	17.50 ± 3.78 ^c
NV	ND	ND	ND	28.60 ± 4.25 ^a	21.70 ± 3.48 ^b	13.50 ± 2.14 ^c
NB	ND	ND	ND	21.10 ± 3.52 ^a	15.30 ± 2.75 ^b	6.80 ± 1.26 ^c
RP	ND	ND	ND	11.30 ± 2.15 ^a	7.40 ± 1.38 ^b	2.90 ± 0.78 ^c

*MN and CAs were scored in 1,000, MI was scored in 10,000 cells in each group. Different letters^(a–d) in the same line are statistically important at $p < 0.05$. ND not determined, DCN dividing cell number, MI mitotic index, FRG fragment, MN micronucleus, UDC unequal distribution of chromatin, SC sticky chromosome, B bridge, NV nucleus with vacuoles, NB nucleus bud, RP reverse polarization.

and leaves of the plant and accumulate there. As a result, by affecting the enzyme systems, it can cause a decrease in root elongation, weight loss, membrane destruction, oxidative stress, loss of biosynthetic activity, and ion leakage in the plant (Chaneva and Petrova 2014). Statistically significant ($p < 0.05$) increases were observed again in all physiological parameters examined in groups V and VI, in which 190 and 380 mg/L doses of GTLE were applied together with paraquat. It was determined that these increases were more pronounced at the dose of 380 mg/L of GTLE. Compared to the paraquat exposed group IV, the germination percentage increased by 21%, root length 5.83 times and weight gain 2.92 times increased in group VI exposed to 380 mg/L dose of GTLE. Although the number of studies on the physiological changes that paraquat application induces in *A. cepa* is quite limited in the literature, there are different studies using different plant species or herbicide varieties. Acar et al. (2015) investigated the physiological toxicity induced by three different doses of paraquat (10, 50, and 100 ppm) in *A. cepa*. Germination percentage, root length, and weight gain were used as indicators of toxicity. As a result, it was reported that Paraquat administration caused a decrease in all physiological parameter values examined in all three doses, and these decreases were even more pronounced at the 100 ppm dose of paraquat. Chaneva and Petrova (2014) investigated the toxicity of paraquat using two different application methods, spraying and adding the herbicide to the feeding medium, using pea and corn plants as indicators. As a result, it was shown that the addition of paraquat to the medium caused more paraquat uptake by the plants compared to the spraying method, and a greater reduction in the growth rates, dry weights, and shoot lengths of the plants. It was also observed that these decreases were higher in corn plant than in pea plant. Çavusoğlu et al. (2011) investigated

the effects of glyphosate herbicide on the physiology of *A. cepa*. As a result, it was determined that glyphosate exposure caused statistically significant decreases in root length, germination percentage, and weight gain depending on the dose. Aydın and Liman (2020) investigated the effects of three different doses of pinoxaden herbicide (1.25, 2.5, and 5.0 mg/L) on *A. cepa* root growth. As a result, pinoxaden significantly reduced root growth depending on the application dose.

Cytogenetic effects

The cytogenetic effects of paraquat and GTLE application are shown in Table 2 and Fig. 1. The highest MI and lowest MN and CA numbers were observed in the control group and groups II and III, which were administered GTLE at doses of 190 and 380 mg/L. The only CAs observed in these groups was the formation of sticky chromosomes. Exposure to paraquat at a dose of 100 mg/L caused a decrease in the MI values of the root tips, and an increase in the number of MN formation and CAs. Compared to the control group, MI decreased approximately 1.46 times (2.81%), the frequency of MN increased approximately 350 times and fragment formation, which is the most observed CAs, increased approximately 70 times in paraquat applied group IV. It was determined that this increase and decrease was statistically significant ($p < 0.05$). MN is a nuclear formation arising from an entire chromosome or chromosome fragments. Similarly, damages such as fragments, sticky chromosomes, and bridges are also caused by damaged DNA and chromosomes. In addition, the main reason for the decrease in the MI is the damages in DNA and chromosomes, the decrease in microtubule and microfilament density, and the deterioration in its distribution. All these cytogenetic changes that occur as a result of

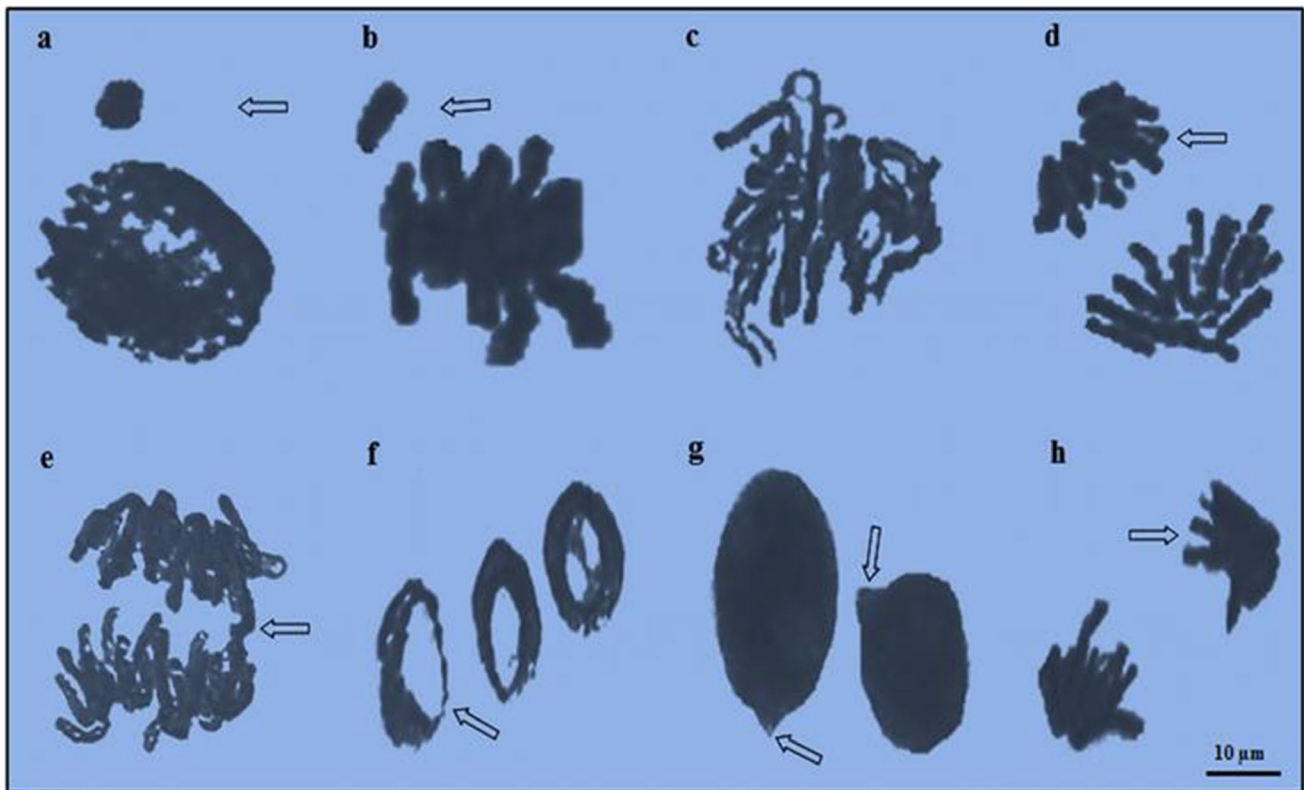


Fig. 1 Chromosomal damages induced by paraquat. **a** MN; **b** fragment; **c** sticky chromosome; **d** unequal distribution of chromatin; **e** bridge; **f** nucleus with vacuoles; **g** nucleus bud; **h** reverse polarization

paraquat exposure can be explained by the direct/direct contact of paraquat with DNA and chromosomes. Vivarelli et al. (2013) reported that paraquat directly induces DNA damage, promoting changes in the splicing patterns of genes involved in cell cycle control, DNA repair, and apoptosis. In addition, there are similar studies in the literature that paraquat can cause DNA damage indirectly through the formation of ROS (Ali et al. 1996). The application of GTLE at two different doses (190 and 380 mg/L) together with paraquat decreased the genotoxic effects of paraquat and caused an improvement in the cytogenetic parameter values examined. It was observed that this improvement was even more pronounced at a dose of 380 mg/L of GTLE. Compared to the paraquat exposed group IV, MI increased approximately 1.31 times (1.66%), the frequency of MN was approximately 1.90 times, and fragment formation approximately 2.30 times decreased in group VI exposed to 380 mg/L dose of GTLE. In the literature, there are different studies investigating the effects of other herbicides on this subject with the studies carried out by our study team regarding the cytogenetic changes promoted by paraquat in the *A. cepa* root tips. Özen et al. (2011) investigated the cytogenetic changes in *A. cepa* root tip cells

by the application of paraquat at three different doses (10, 50, and 100 ppm). MI, MN, and CAs were used as indicators of cytotoxicity. As a result, a decrease in MI and an increase in the numbers of MN and CAs were reported depending on the dose of paraquat. Paraquat administration promoted CAs in the form of bridge, vagrant, and scattered phase formations. Similarly, Acar et al. (2015) investigated the genotoxicity induced by 10, 50, and 100 ppm paraquat doses in *A. cepa* root tip cells. As a result, it was determined that paraquat administration dose-dependently increased the frequency of MN, promoted CAs such as fragment, spindle yarn abnormality, abnormal polarization, sticky chromosome, bridge, c-mitosis, binucleated cell, and unequal distribution of chromatin. Gömürgen (2000) investigated the genotoxicity caused by 7 different doses of 2,4-D isooctylester 48% (Esteran 48) herbicide, ranging from 50 to 50,000 ppm during 3, 6, 12, and 24 h in *A. cepa* root tip cells. As a result, it was observed that Esteran 48 caused a decrease in MI, depending on the dose and duration applied, and also caused CAs in the form of c-metaphase, sticky chromosome, bridge, tetraploid cells, vagrant chromosome, tripolar anaphases-telophases, and MN.

Table 3 Effects of paraquat and GTLE on selected biochemical parameters

Groups	MDA ($\mu\text{M g}^{-1}$ FW)	SOD (U mg^{-1} FW)	CAT ($\text{OD}_{240\text{nm}} \text{min g}^{-1}$ FW)
I	$5.50 \pm 1.75^{\text{d}}$	$60.50 \pm 5.18^{\text{d}}$	$0.35 \pm 0.13^{\text{d}}$
II	$5.30 \pm 1.66^{\text{d}}$	$58.70 \pm 5.12^{\text{d}}$	$0.32 \pm 0.11^{\text{d}}$
III	$5.10 \pm 1.52^{\text{d}}$	$59.30 \pm 5.24^{\text{d}}$	$0.30 \pm 0.10^{\text{d}}$
IV	$14.20 \pm 2.63^{\text{a}}$	$150.20 \pm 9.14^{\text{a}}$	$1.58 \pm 0.85^{\text{a}}$
V	$11.80 \pm 2.14^{\text{b}}$	$130.10 \pm 8.18^{\text{b}}$	$1.22 \pm 0.63^{\text{b}}$
VI	$8.00 \pm 1.84^{\text{c}}$	$94.40 \pm 6.52^{\text{c}}$	$0.96 \pm 0.51^{\text{c}}$

*Data are given as mean \pm SD ($n=10$). Different letters^(a-d) in the same column are statistically important at $p < 0.05$.

Biochemical effects

The effects of paraquat and GTLE application on selected biochemical parameters are shown in Table 3. The lowest MDA, SOD, and CAT levels were measured in group I, group II, and group III. Paraquat administration caused statistically significant ($p < 0.05$) increases in levels of MDA, SOD, and CAT. Compared to the control group, MDA level increased 2.58 times, SOD activity 2.48 times, and CAT activity 4.51 times increased in group IV exposed to paraquat at a dose of 100 mg/L. MDA is a small and reactive organic molecule containing two aldehyde groups and three carbon molecules. MDA formation can be induced by ROS attack (non-enzymatic) or lipoxygenase activity (enzymatic). Determination of lipid peroxidation in the cell is usually done by measuring aldehydes such as MDA. The amount of MDA is used as an indicator of damage to plant membranes, since most of the MDA originates from polyunsaturated fatty acid lipid peroxidation in plant membranes in response to oxidative stress. However, in recent years, some scientists have suggested that the increase in MDA in the cell plays an important role in cell protection by activating the regulatory genes. In other words, it has been suggested that although MDA is an important indicator of cell damage, it may also function as a protection mechanism that prevents further progression of cell damage. Genetic evidence has shown that membranes rich in polyunsaturated fatty acids act as antioxidants that trap ROS, thereby decreasing the cell damages. In this process, it continuously produces lipid degradation products such as MDA. Therefore, MDA is considered both a toxic (harmful) molecule and a gene activator (protector) (Morales and Munne Bosch 2019). In our study, the main reason for the increase in MDA levels in root cells treated with paraquat is that paraquat promotes the formation of ROS, which in turn attacks the polyunsaturated fatty acids in the membranes of the stem cells and increases MDA production. Because epidermis and cortex cell damage obtained from microscopic examination of root tip cells

of *A. cepa* also supports our view. ROS can be composed of non-radical species such as hydrogen peroxide and free radical species such as superoxide (O_2^-) or hydroxyl radical ($\cdot\text{OH}$). In this context, some studies have revealed that the mechanism of Paraquat toxicity is related to a redox-cyclic reaction that produces singlet oxygen, superoxide anion, and other free radicals and leads to lipid peroxidation of cell membranes (Ranjbar 2014).

Antioxidant enzymes are a set of proteins found in the cellular environment, regulating antioxidants in the process of scavenging free radicals and facilitating their work. The main function of these enzymes is to facilitate the electron-donating mechanisms of antioxidants. Two of the most important antioxidant enzymes in the biological system are CAT and SOD. Thanks to these two enzymes, organisms can deal with hydrogen peroxide, superoxide ion (O_2^-) and hydroxyl radicals ($\cdot\text{OH}$). Environmental negativities such as drought, low–high temperatures, macro-nutrient deficiency, exposure to heavy metals or pesticides in plants encourage the formation of ROS, and CAT and SOD activity in the cell increases in order to combat the formed ROS (Stephenia et al. 2020). In this study, the increase in CAT and SOD activities in root cells treated with paraquat was attributed to the increase in CAT and SOD enzyme activities during the process of paraquat promoting the formation of ROS in stem cells and clearing these ROS from the cell. Hu et al. (2018) reported that paraquat, as an electron acceptor, induces the formation of a large number of ROS after entering the cell, thereby causing cell damage. The application of GTLE at doses of 190 and 380 mg/L together with paraquat caused a decrease in paraquat toxicity and again caused a statistically significant decrease in the investigated biochemical parameter values, although not as much as the control group. In addition, these decreases were observed to be more pronounced at the dose of 380 mg/L of GTLE. Compared to group IV in which paraquat was administered, the MDA level decreased 1.78 times, SOD activity 1.59 times, and CAT activity 1.65 times in group VI administered 380 mg/L dose of GTLE. Although the number of studies investigating the biochemical toxicity of paraquat in *A. cepa* root cells is quite limited in the literature, there are different studies investigating the biochemical changes caused by other herbicides in *A. cepa* and other plant materials. Özen et al. (2011) investigated the biochemical toxicity induced by paraquat at 10, 50, and 100 ppm doses in *A. cepa* root tip cells. CAT, SOD, and MDA levels were used as indicators of biochemical toxicity. As a result, statistically significant increases in CAT, SOD, and MDA levels were reported depending on the dose of paraquat administered. Çavuşoğlu et al. (2011) reported that glyphosate increased MDA levels in *A. cepa* root. Çanakçı-Gülengül and Karabulut (2020) investigated

Table 4 Protective role of GTLE against paraquat-induced anatomical damage

Groups	UVT	ECD	CCD	FCN
I	-	-	-	-
II	-	-	-	-
III	-	-	-	-
IV	++	+++	++	+++
V	+	++	+	++
VI	-	+	-	+

*UVT unclearly vascular tissue, ECD epidermis cell deformation, CCD cortex cell deformation, FCN flattened cell nucleus. (-) no damage, (+) little damage, (++) moderate damage, (+++) severe damage.

the biochemical changes induced by atrazine and metolachlor herbicides at 100 μM, 300 μM, and 1000 μM doses in *Triticum aestivum* L. (wheat) varieties. As a result, it was observed that both herbicides caused an increase in MDA levels and SOD activity, and a decrease in CAT activity, depending on the dose.

Anatomical effects

The anatomical damages induced by paraquat in *A. cepa* root tip meristematic cells and the protective effect of GTLE against these damages is shown in Table 4 and Fig. 2. Under the light microscope, no damage was observed in the root tip meristematic cells of the bulbs in the control group and groups II and III, which two different doses (190 and 380 mg/L) of GTLE were applied. In root tip meristematic cells of the bulbs in group IV exposed to paraquat at a dose of 100 mg/L were determined damages such as flattened cell nuclei, unclearly vascular tissue, epidermis, and cortex cell deformation. This suggests that these damages may have occurred due to the defense mechanisms developed by *A. cepa* exposed to paraquat to minimize herbicide toxicity. Under the light microscope, it was observed that the roots of *A. cepa* exposed to paraquat increased the number and order of the epidermis and cortex cells in order not to take the herbicide inside. Since these increases will increase the contact and pressure of the cells with each other, the formation of damages such as deformation of cell and the cell

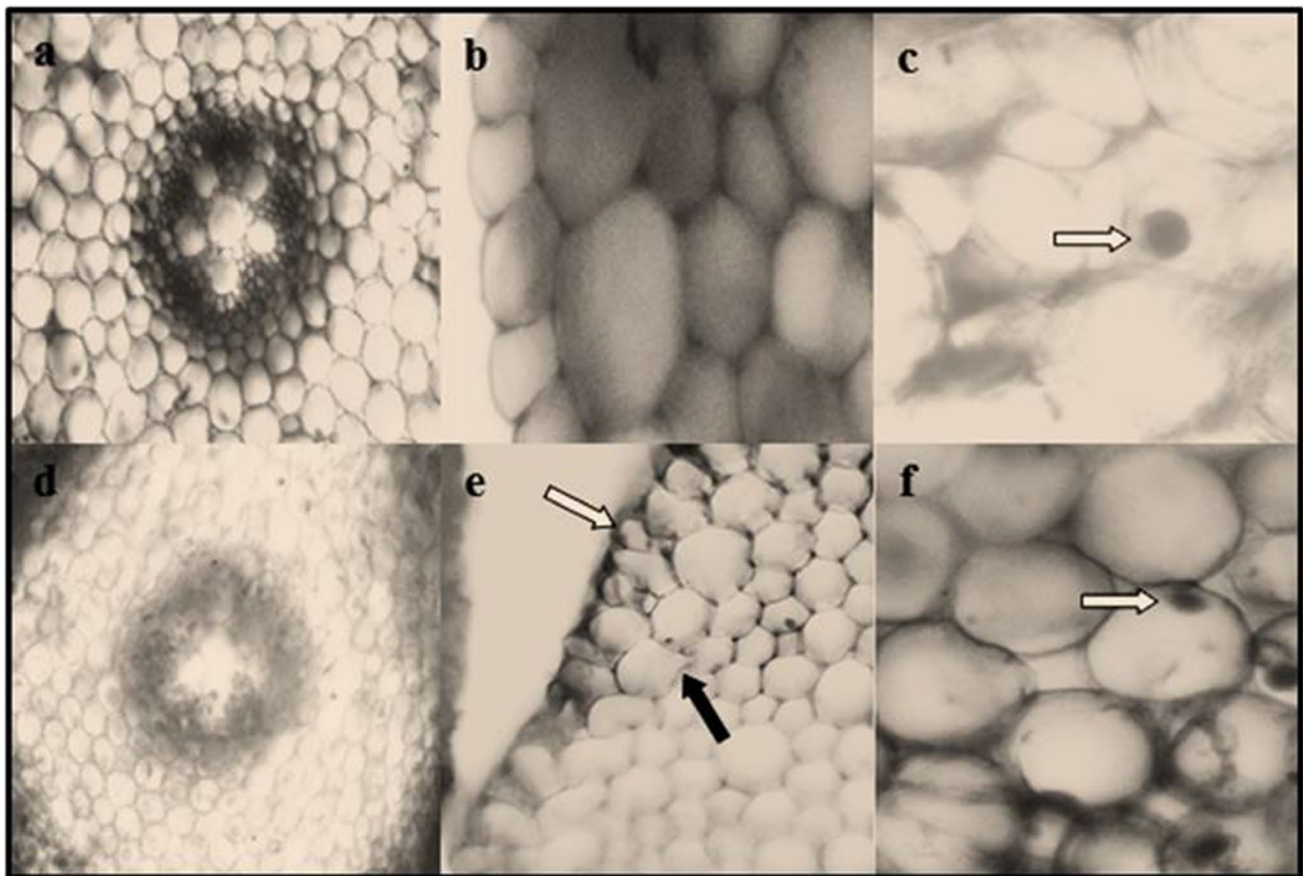


Fig. 2 Anatomical damages induced by paraquat in root tip cells. **a** Vascular tissue normal appearance (×50), **b** epidermis cells normal appearance (×200), **c** cell nucleus normal appearance-oval (×500),

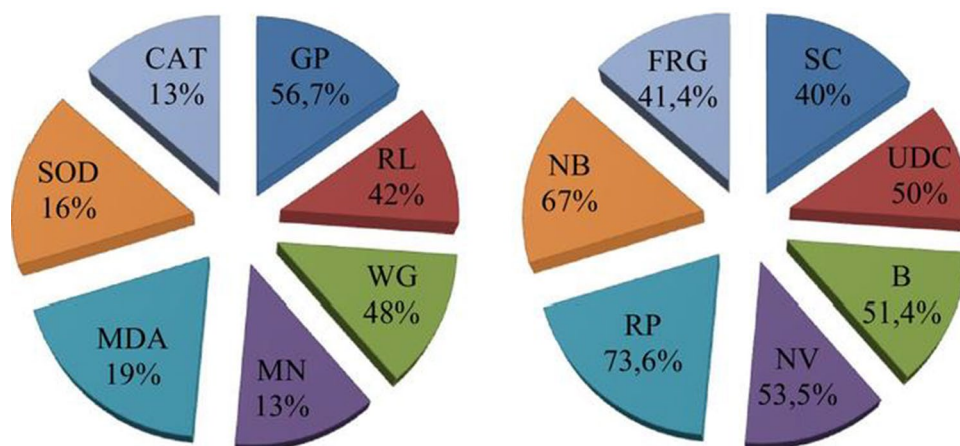
unclearly **d** vascular tissue (×50), **e** epidermis (white arrow,×200) and cortex (black arrow,×200) cell deformation, **f** flattened cell nucleus (×500)

nucleus may be inevitable. There is a lot of information in the literature, that plants develop various defense mechanisms against pesticide toxicity, such as the activation of the antioxidant defense system, various physiological and morphological changes (trichomes, increase in the number of leaves and roots, increase in the number of cells and layers, etc.), release of different chemical defense compounds (alkaloids, terpenoids, phenolic compounds, etc.), accumulation of toxic substances and thickening of the cortex cell wall in certain regions (Mithöfer and Maffei 2016; Sharma et al. 2019; Kalefetoğlu Macar et al. 2021). The application of GTLE at two different doses (190 and 380 mg/L) together with Paraquat reduced the toxicity of Paraquat and caused an improvement in the severity of the damages observed in root tip meristem cells. It was observed that this improvement was even more pronounced at a dose of 380 mg/L of GTLE. Such that, in Group VI, which was applied of 380 mg/L dose of GTLE unclearly vascular tissue and cortex cell deformation damages were no longer observed. Although the number of studies investigating the anatomical changes caused by paraquat in *A. cepa* root cells is very limited in the literature, there are similar studies investigating the effect of other herbicides. Acar et al. [6] investigated the anatomical changes induced by paraquat application at 10, 50, and 100 ppm doses in *A. cepa* root tip meristematic cells. Anatomical damages such as flattened cell nuclei, necrosis, unclearly vascular tissue, accumulation of some substances in the vascular tissue and cell deformation have been reported in root tip meristematic cells, depending on the dose of paraquat applied. Çavuşoğlu et al. (2011) investigated the anatomical damages caused by three different doses of glyphosate herbicide in *A. cepa* roots. In conclusion, light micrographs showed that glyphosate exposure induced anatomical damages such as unclearly vascular tissue, flattened cell nucleus, cell deformation and binucleated cell in root tip cells at all three doses. Ogeleka et al. (2016) investigated the phytotoxic effects induced by 0.625, 1.25, 2.5, 5, and 10 mg/L doses of Roundup (glyphosate) herbicide in *A. cepa* bulbs. As a result, it was determined that growth slowed down, bulb deformation, tissue, and root damage occurred, especially at 10 mg/L dose which highest dose of Roundup.

In the last 10 years, various herbal extracts have been used to reduce pesticide toxicity in scientific studies carried out on the toxic effects of pesticides. Some of these products can be listed as *Ginkgo biloba* L., lycopene, carotene, grape seed, nettle, echinacea, blackberry, and green coffee. In this study, GTLE was used to reduce the physiological, cytogenetic, biochemical, and anatomical toxicity induced by paraquat herbicide in *A. cepa*. One hundred milligrams per liter paraquat exposure caused adverse changes in all selected cytogenetic, biochemical, physiological, and anatomical parameters in *A. cepa*. The application of GTLE at two different doses (190 and 380 mg/L), on the other hand, reduced

the toxicity caused by paraquat and caused an improvement in all investigated parameters, depending on the dose. This protective role of GT has been attributed to its antioxidant activity. Because GT is a beverage rich in flavonoids, flavonols (myricetin, kaemferol, quercetin, chlorogenic acid, coumoylquinic acid, and theogallin), polyphenols, caffeine, minerals (F, Mn, Cr, Se, Ca, Mg, and Zn), vitamins, amino acids, and carbohydrates. GT is mainly composed of catechin flavonoids. For example, GT contains higher catechins than apples, red grapes, and wine. EGC, EC, EKG, and EGCG are the main catechins found in GT. All these substances in its content give GT an antioxidant property. Thus, by increasing the antioxidant power of the organism, GT consumption provides benefits in many processes such as clearing ROS from the cell, preventing cancers, eliminating cardiovascular disorders, regulating cholesterol, delaying aging, and reducing inflammation (Prasanth et al. 2019). There are similar studies in the literature on the antioxidant role of GT extract. Yapar et al. (2009) investigated the protective role of GT extract against nephrotoxicity induced by the cancer drug cisplatin in albino mice. As a result, it was determined that cisplatin administration caused an increase in kidney MDA, creatinine, and blood urea nitrogen (BUN) levels and a decrease in kidney glutathione (GSH) levels. The administration of GT extract with cisplatin caused an improvement in nephrotoxicity caused by cisplatin, resulting in a decrease in kidney MDA, BUN and creatinine levels, and an increase in kidney GSH levels again. Yalçın et al. (2015) investigated the protective role of GT extract against toxicity induced by formaldehyde. As a result, exposure to formaldehyde caused an increase in ALP, ALT, and AST enzyme activities and in MDA levels and a decrease in GSH levels. It also promoted hepatocyte degeneration and necrosis damages in the liver. GT extract application provided significant protection against formaldehyde-induced toxicity. Thanks to this protection, necrosis damages, hepatocyte degeneration, ALP, ALT, and AST enzyme activities, and a decrease in MDA levels and an increase in GSH levels were observed again. Akgündüz et al. (2019) investigated the protective role of GT extract against physiological and genetic toxicity caused by chronic formaldehyde exposure in albino mice. Body weight and organ weights were used as indicators of physiological toxicity, and the frequency of MN in leukocyte cells was used as indicators of genetic toxicity. As a result, it was determined that formaldehyde toxicity caused a decrease in body weight, liver, and kidney organ weights, and an increase in the frequency of leukocyte MN. Feeding rats with GT extract together with formaldehyde caused a significant improvement in formaldehyde toxicity, again an increase in body and organ weight values, and a decrease in leukocyte MN frequency. Sağır et al. (2013) investigated the protective role of GT extract against the physiological and genetic changes induced by 1,4 dioxane exposure. Body

Fig. 3 RE of GTLE. GP: germination percentage, RL: root length, WG: Weight gain, MDA: malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, MN: micronucleus, FRG: fragment, SC: sticky chromosome, UDC: unequal distribution of chromatin, B: bridge, NV: nucleus with vacuoles, NB: nucleus bud, RP: reverse polarization



weight was used as indicators of physiological toxicity; MI, MN, and CAs were used as indicators of genotoxicity. In conclusion, exposure to 1,4 dioxane caused a decrease in body weight and MI, and an increase in the frequency of MN in erythrocyte and buccal mucosal epithelial cells. In addition, 1,4 dioxane exposure induced CAs such as fracture, acentric, dicentric, and gap in bone marrow cells. GT extract application reduced the toxic effects of 1,4 dioxane exposure and caused an improvement in all the parameters examined. An increase in body weight and MI values, and a decrease in the number of MN and CAs were detected again. It has also been reported that these improvements observed in all investigated parameter values were even more pronounced at a dose of 100 mg/kg b.w of GT extract.

Recovery effects of GTLE

The protective effect of 380 mg/L GTLE against all abnormalities induced by paraquat is given in Fig. 3. GTLE exhibited protective properties against each abnormality at different rates. It exhibited a RE in the range of 42–56.7% compared to the physiological parameters such as root length, germination percentage and weight gain. The highest protective effect in biochemical parameters was determined as 19% against MDA level. While it provided a protection in the range of 40–73.6% against CAs, it exhibited the highest protective effect against reverse polarization. All these protective properties are related to the multibiological functions and phochemical content of GLTE.

Conclusion

In this study, the toxicity induced by paraquat, one of the most commercially sold herbicides in the world, in *A. cepa* and the protective role of GTLE against this toxicity were investigated with the help of cytogenetic, physiological, biochemical, and

anatomical parameters. The number of studies investigating the toxic effects of paraquat on *A. cepa* is quite limited in the literature. On the other hand, in current studies, paraquat toxicity has not been addressed in all its aspects with the help of cytogenetic, physiological, biochemical, and anatomical parameters. In addition, to date, there is no other study investigating the protective role of GTLE against paraquat toxicity in *A. cepa*. Considering all these issues, this study is quite original in terms of introducing new information to the literature. As a result of the research, it was determined that exposure to paraquat at a dose of 100 mg/L caused toxicity in terms of all parameters investigated in *A. cepa*. GTLE application at doses of 190 and 380 mg/L, on the other hand, induced a decrease in paraquat toxicity, thanks to the antioxidant property of GT, and caused an improvement in all investigated parameter values. In addition, it was determined that this improvement was even more pronounced at the dose of 380 mg/L of GTLE. For this reason, GT is a beverage that must be included in the daily diet as an antioxidant nutrient in reducing or protection from the toxic effects of environmental chemical agents such as pesticides.

Author contribution All authors (Ferhat Yirmibeş, Emine Yalçın, Kültiğın Çavuşođlu) contributed to the study conception and design. All authors read and approved the final manuscript.

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

Declarations

Ethics approval and consent to participate The authors confirm that the manuscript has been read and approved by all authors. The authors declare that this manuscript has not been published and not under consideration for publication elsewhere.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

- 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(3):343–351. <https://doi.org/10.1508/cytologia.80.343>
- Akash MM, Pawade UV, Nikam AV (2018) Classification of pesticides: a review. *Int J Res Ayurveda Pharm* 9(4):144–150. <https://doi.org/10.7897/2277-4343.09413>
- Akgündüz MÇ, Çavuşoğlu K, Yalçın E, Yapar K, Acar A (2019) Protective role of green tea extract against toxicity caused by chronic formaldehyde exposure. *Black Sea 1st International Multidisciplinary Scientific Studies Congress, Giresun-Turkey*, pp 185–190
- Ali S, Jain SK, Abdulla M, Athar M (1996) Paraquat induced DNA damage by reactive oxygen species. *IUBMB Life* 39(1):63–67. <https://doi.org/10.1080/15216549600201061>
- Atik M, Karagüzel O, Ersoy S (2007) Effect of temperature on germination characteristics of *Dalbergia sissoo* seeds. *Mediterr Agric Sci* 20:203–208
- Aydın G, Liman R (2020) Cytogenotoxic effects of pinoxaden on *Allium cepa* L. roots. *J Appl Genet* 61:349–357. <https://doi.org/10.1007/s13353-020-00560-w>
- Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal Biochem* 44(1):276–287. [https://doi.org/10.1016/0003-2697\(71\)90370-8](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
- Chaneva G, Petrova D (2014) Effect of method of applying the herbicide paraquat on pea and maize. *Oxid Commun* 37(4):1090–1102
- Çanakçı-Gülcengül S, Karabulut F (2020) The biochemical changes caused by metolachlor and atrazine on wheat (*Triticum aestivum* L.) varieties. *Prog Nutr* 22(3):1–8. <https://doi.org/10.23751/pn.v22i3.8336>
- Çavuşoğlu K, Kurt D, Yalçın E (2020) A versatile model for investigating the protective effects of *Ceratonia siliqua* pod extract against 1,4-dioxane toxicity. *Environ Sci Pollut Res* 27:27885–27892. <https://doi.org/10.1007/s11356-020-08545-2>
- Çavuşoğlu K, Yalçın E, Türkmen Z, Yapar K, Çavuşoğlu K, Çiçek F (2011) Investigation of toxic effects of the glyphosate on *Allium cepa*. *J Agric Sci* 17(2):131–142. https://doi.org/10.1501/Tarimbil_0000001165
- 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(1):65–75. [https://doi.org/10.1016/s1383-5718\(02\)00249-8](https://doi.org/10.1016/s1383-5718(02)00249-8)
- Gömürgen AN (2000) Cytological effect of the herbicide 2, 4-D isooctylester 48% on root mitosis of *Allium cepa*. *Cytologia* 65(4):383–388. <https://doi.org/10.1508/cytologia.65.383>
- Gupta J, Siddique YH, Beg T, Ara G, Afzal M (2009) Protective role of green tea extract against genotoxic damage induced by anabolic steroids in cultured human lymphocytes. *Biol Med* 1(2):87–99
- Gündüz A, Yalçın E, Çavuşoğlu K (2021) Combined toxic effects of aflatoxin B₂ and the protective role of resveratrol in Swiss albino mice. *Sci Rep* 11(1):1–14. <https://doi.org/10.1038/s41598-021-95879-7>
- Hashmi I, Khan AD (2011) Adverse health effects of pesticide exposure in agricultural and industrial workers of developing country. In: *Pesticides-the impacts of pesticides exposure*, pp 156–178. <https://doi.org/10.5772/13835>
- Hu S, Qiao C, Yuan Z, Li M, Ye J, Ma H, Zhang J (2018) Therapy with high-dose long-term antioxidant free radicals for severe paraquat poisoning: a pilot study. *Exp Ther Med* 16(6):5149–5155. <https://doi.org/10.3892/etm.2018.6823>
- Kalefetoğlu Macar T, Macar O, Yalçın E, Çavuşoğlu K (2021) Preventive efficiency of cornelian cherry (*Cornus mas* L.) fruit extract in diniconazole fungicide-treated *Allium cepa* L. roots. *Sci Rep* 11:2534. <https://doi.org/10.1038/s41598-021-82132-4>
- Mithöfer A, Maffei ME (2016) General mechanisms of plant defense and plant toxins. *Plant Toxins*, Springer AG, Switzerland.
- Mohabbulla Mohib M, Fazla Rabby SM, Paran TZ et al (2016) Protective role of green tea on diabetic nephropathy—a review. *Cogent Biol* 2(1):1248166. <https://doi.org/10.1080/23312025.2016.1248166>
- Morales M, Munné-Bosch S (2019) Malondialdehyde: facts and artifacts. *Plant Physiol* 180(3):1246–1250. <https://doi.org/10.1104/pp.19.00405>
- Ogeleka DF, Okieimen FE, Ekpudi FO, Tudararo-Aherobo LE (2016) Short-term phyto-toxicity consequences of a nonselective herbicide glyphosate (Roundup™) on the growth of onions (*Allium cepa* Linn). *Afr J Biotechnol* 5(18):740–744. <https://doi.org/10.5897/AJB2014.14355>
- Oyetaş White P, Tribout H, Baron E (2012) Protective mechanisms of green tea polyphenols in skin. *Oxid Med Cell Longev* 2012:560682. <https://doi.org/10.1155/2012/560682>
- Özen E, Çiçek F, Gür B, Aydın N, Akıncı B, Topal M, Keser G, Çavuşoğlu K (2011) The effects of Paraquat on some cytotoxic and biochemical parameters in *Allium cepa*. *Firat Uni J Sci* 23(2):117–124
- Prasanth MI, Sivamaruthi BS, Chaiyasut C, Tencomnao T (2019) A review of the role of green tea (*Camellia sinensis*) in anti-photoaging, stress resistance, neuroprotection, and autophagy. *Nutrients* 11(2):474. <https://doi.org/10.3390/nu11020474>
- Ranjbar A (2014) Evidence of oxidative damage in paraquat toxicity. *Zahedan J Res Med Sci* 16(12):1–8
- Reddy KBK, Jeevanalatha M, Lakshman M, Rani MU (2019) The toxic effects of paraquat (PQ) on body weights and haematological parameters in male albino wistar rats and its amelioration with vitamin C. *Int J Curr Microbiol App Sci* 8(11):314–320. <https://doi.org/10.20546/ijcmas.2019.811.039>
- Sağır S, Çavuşoğlu K, Yapar K (2013) Investigation of physiological and genotoxic effects of 1,4 dioxane on swiss albino mice. *EÜF-BED* 6(2):145–155
- Sharma A, Kumar V, Thukral AK, Bhardwaj R (2019) Responses of plants to pesticide toxicity: an overview. *Planta Daninha* 37:e019184291. <https://doi.org/10.1590/S0100-83582019370100065>
- Staykova TA, Ivanova EN, Velcheva IG (2005) Cytogenetic effect of heavy-metal and cyanide in contaminated waters from the region of southwest Bulgaria. *J Cell Mol Biol* 4:41–46
- Stephens S, Chang YP, Gnanasekaran A, Esa NM, Gnanaraj C (2020) An insight on superoxide dismutase (SOD) from plants for mammalian health enhancement. *J Funct Foods* 68:103917. <https://doi.org/10.1016/j.jff.2020.103917>
- Tosun N, Karabay NÜ, Sayım F (2001) Pesticide usage and their potential adverse impacts on living organisms. *Anadolu J AARI* 11(1):113–125
- Tu M, Hurd C, Randall JM (2001) *Weed control methods handbook: tools & techniques for use in natural areas*. The Nature Conservancy, Available from: <https://www.invasive.org/gist/products/handbook/methods-handbook.pdf>. Accessed 01/09/2021
- Unyayar S, Celik A, Cekic FO, Gozel 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>
- Wei QX (2004) Mutagenic effects of chromium trioxide on root tip cells of *Vicia faba*. *J Zhejiang Univ Sci A* 12(5):1570–1576. <https://doi.org/10.1631/jzus.2004.1570>
- Vivarelli S, Lenzen SC, Ruepp MD et al (2013) Paraquat modulates alternative pre-mRNA splicing by modifying the intracellular

- distribution of SRPK2. *PLoS ONE* 8(4):e61980. <https://doi.org/10.1371/journal.pone.0061980>
- Yalçın E, Çavuşoğlu K, Çiçek F, Demirtaş G, Taşlı B (2015) Histopathological and biochemical changes in Swiss albino mice induced by formaldehyde: protective effect of green tea extract. *Cytologia* 80(4):467–473. <https://doi.org/10.1508/cytologia.80.467>
- Yapar K, Çavuşoğlu K, Oruç E, Yalçın E (2009) Protective effect of royal jelly and green tea extracts effect against cisplatin-induced nephrotoxicity in mice: a comparative study. *J Med Food* 12(5):1136–1142. <https://doi.org/10.1089/jmf.2009.0036>
- Zacharia JT (2011) Identity, physical and chemical properties of pesticides, pesticides in the modern world-trends in pesticides analysis. IntechOpen, Available from: <https://www.intechopen.com/chapters/20983>. <https://doi.org/10.5772/17513>. Accessed 12/09/2021
- Zou J, Yue J, Jiang W, Liu D (2012) Effects of cadmium stress on root tip cells and some physiological indexes in *Allium cepa* var *agrogarum* L. *Acta Biol Cracov Bot* 54(1):129–141. <https://doi.org/10.2478/v10182-012-0015-x>. Accessed 22 Sept 2021
- Zacharia JT (2011) Identity, physical and chemical properties of pesticides, pesticides in the modern world - trends in pesticides analysis. Margarita Stoytcheva. <https://doi.org/10.5772/17513>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.