Vitae
School of Pharmaceutical and Food Sciences
University of Antioquia

ORIGINAL ARTICLE
Published 23 September 2024
Doi: https://doi.org/10.17533/udea.vitae.v31n2a352429

Evaluation of toxic effects of rabeprazole sodium on the plant-based eukaryotic test models

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JOURNAL VITAE
School of Pharmaceutical and Food Sciences
ISSN 0121-4004 | ISSNe 2145-2660
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Received: 24 January 2024
Accepted: 15 August 2024
Published: 23 September 2024

ABSTRACT

Background: Rabeprazole (RPZ), a widely used proton pump inhibitor, is known to have toxic effects on human beings. **Objective**: To evaluate the toxic effects of RPZ sodium (RPZ-Na) using plant-based eukaryotic test systems. **Methods**: The toxic effect of RPZ-Na (0.025-0.4 mM) was evaluated on *Allium cepa, Allium sativum*, and *Cicer arietinum* at different exposure times using $CuSO_4$ as a reference standard. **Results**: RPZ-Na concentration-dependently reduced the root length of *A. cepa* and *A. sativum*, as well as the shoot and root lengths of *C. arietinum*. RPZ-Na at 0.1 to 0.4 µg/mL and at 48 h exposure time exerted toxic effects on the tested systems. **Conclusions**: RPZ-Na exerted a concentration- and time-dependent toxic effect on *A. cepa, A. sativum*, and *C. arietinum*. Therefore, it is important to take adequate precautions during its long-term use.

Keywords: Rabeprazole sodium; *Allium cepa; Allium sativum; Cicer arietinum;* Toxicity monitoring tools

RESUMEN

Antecedentes: El rabeprazol (RPZ), un inhibidor, de la bomba de protones del estómago, ampliamente utilizado; sin embzrgo, tiene efectos tóxicos en los seres humanos. **Objetivo**: Evaluar los efectos tóxicos del RPZ sódico (RPZ-Na) Utilizando plantas como modelos eucariotas para evaluación de toxicidad. **Métodos**: El efecto tóxico de RPZ-Na (0,025-0,4 mM) en *Allium cepa, Allium sativum* y *Cicer arietinum* en diferentes tiempos de exposición utilizando $CuSO_4$ como estándar de referencia. **Resultados**: RPZ-Na, de forma dependiente de su concentración, redujo la longitud de raíces de *A. cepa* y *A. sativum*, así como el tamaño de los brotes y la raíz de *C. arietinum*. RPZ-Na a 0,1 a 0,4 µg/mL y a 48 h de tiempo de exposición ejerció efectos tóxicos en los sistemas de prueba. **Conclusiones**: RPZ-Na ejerció un efecto tóxico dependiente de la concentración y el tiempo de exposición en *A. cepa, A. sativum* y *C. arietinum*. Por lo tanto, es importante tomar precauciones adecuadas durante su uso a largo plazo.

Palabras clave: Rabeprazol sódico; *Allium cepa; Allium sativum*; *Cicer arietinum;* Herramientas de monitoreo de toxicidad

INTRODUCTION

Rabeprazole (RPZ), a gastric proton pump inhibitor (PPI), inhibits H^+/K^+-ATPase activity in gastric parietal cells. Thereby reducing the overall acid secretion and providing an anti-secretory effect (1). It is used in peptic ulcers and gastro-esophageal reflux disease (GERD) as it can inhibit the overproduction of stomach acid (2). RPZ is also used in multiple endocrine adenomas and systemic mastocytosis (3), associated with other anti-*Helicobacter pylori* medications (4) (BNF, 2018) to eradicate the bacterium and treat hypersecretory conditions (2, 3).

The US Food and Drug Administration (FDA) approved RPZ (2), although it has some common side effects, including constipation, feeling weak, and throat inflammation. Moreover, it can cause osteoporosis, low serum magnesium, *Clostridium difficile* infection, and pneumonia (2). On the other hand, its use during pregnancy and lactation is controversial (5). Drug toxicity refers to the harmful effects that a drug can have on the body (6). The median lethal dose (LD_{50}) value of RPZ in rats is 2.4215 mol/kg (7). It seems RPZ is well tolerated in animals. Generally, potent toxic drugs are not approved for use on humans or animals. Thus, analysis of the safety profile of a particular drug is crucial. A study reported that RPZ (0.1 and 0.2 mM) has cytotoxic effects on several human gastric cancer cell lines (8). This suggests that RPZ has a cytotoxic profile. Drugs with cytotoxicity may cause health hazards. This is because these drugs are irritating and can also produce local harmful effects on specific organs (e.g., skin and eyes) (9).

Due to their health effects, as they contain a specific aroma and many important bioactive chemical compounds, including sulfur compounds and flavonoids (10), many species of the genus *Allium* are commonly used as foods and traditional medicines. *Allium cepa* is a vegetable that is the most widely cultivated species in the genus *Allium*. *A. cepa* has been extensively used to evaluate the toxicogenetic effects (e.g., toxicity, cytotoxicity, genotoxicity, and mutagenicity) of a wide variety of substances. It is more sensitive than others because the onion roots are sensitive to many toxic molecules. Thus, it plays an important role in biomonitoring (11). The *A. cepa* test model is rapid and precise, allows the assessment of several endpoints (e.g., chromosome aberrations, micronuclei formation, mitotic index), and helps us to evaluate the toxic effects of various substances for environmental monitoring (12, 13).

Plants and their seeds, when exposed to a high concentration of toxic substances in soil or culture media, exhibit toxicity symptoms such as a decrease in height (14), inhibition of seed germination, decrease in tillering, reduction in root or shoot growth, decrease in fruit and grain yield (15, 16) and even death (17). *Cicer arietinum* seeds, widely cultivated in Bengal, are a major food source in the region. The *in vitro* toxic effects of chemical substances can be studied by knowing the germination and seedling profiles of *C. arietnum* (18).

Considering the facts mentioned above, the current study aimed to evaluate the toxic effects of rabeprazole sodium on *A. cepa, A. sativum,* and *C. arietinum.*

METHODS

Collection of test systems

Medium-sized onions (*A. cepa*), garlic (*A. sativum*), and fresh brown *C. arietinum* were purchased from the local market in Gopalganj district, Bangladesh, and subjected to toxicity analysis.

Reagents and chemicals

Rabeprazole sodium (RPZ-Na) was obtained from Aristopharma Ltd., Bangladesh, while $CuSO_4$ was purchased from Merck, India.

Preparation of test concentrations

Test concentrations were selected according to previous studies. Gu et al. (8) performed an *in vitro* cytotoxicity analysis of RPZ on several human gastric cancer cells at 0.1 and 0.2 nM. Yaşar et al. (19) carried out a study on human pylorus muscle cells at 0.001 to 1 mM RPZ. Therefore, this study evaluated the toxic effects of RPZ-Na on *A. cepa, A. sativum,* and *C. arietinum* test systems at five concentrations: 0.025, 0.05, 0.1, 0.2, and 0.4 mM. RPZ-Na was dissolved in distilled water.

Toxic effects on root growth profile

A. cepa test

The outer layer(s) and the budding parenchyma of the central crown were carefully removed to promote the root growth of *A. cepa*. A small, circular incision was also made. Then, the bulbs were rinsed with tap water for 30 minutes. The root portion of each onion was soaked in distilled

water in a plastic container (capacity: 15–20 mL) for the first 24 h at 25 ± 1 °C in the dark. *A. cepa* samples (five for each concentration) with fair root growth were reassigned to the NC (negative control, distilled water) and test samples containing the concentrations mentioned above of RPZ-Na for a further 72 h. After every 24 hours, the number and length of the 10 longest roots were counted for each bulb. The root length was measured. The toxicity of the RPZ-Na was determined by evaluating the root growth inhibition profile of the test sample or PC (positive control treated with 38 µM $CuSO_4$) during inspection of the NC group (20). The half maximal inhibitory concentration (IC_{50}) was also determined for the RPZ-Na at each exposure time by non-linear regression analysis using Graph Pad Prism software, as mentioned below.

A. sativum test

The outer cloves of *A. sativum* were collected. Both the inner and outer peels of each clove were carefully removed. Similarly, the budding parenchyma from the central crown of each clove was carefully removed by making a small spheroid incision to facilitate root growth. The cloves were washed with tap water for 5 min, and the root portion was soaked in the test sample or control plastic containers (capacity: 15–20 mL) up to 72 h at 28 ± 1 °C in the dark. After every 24 hours, the number and length of the 10 longest roots were counted and measured for each bulb. The root length was calculated similarly to the *A. cepa* plant test and measured to determine the toxic effects of the RPZ-Na and controls. Distilled water and 38 µM $CuSO_4$ were used as NC and PC groups, respectively. The IC_{50} values were calculated as mentioned above.

Toxic effects on germination profile

C. arietinum test

The chickpea seeds (60 days old) were rinsed three times and soaked for 10 minutes in distilled water. Seed germination was tested on moist sanitary napkin tissue papers. For this purpose, two-layered seedling beds were prepared with small pieces of tissue paper on clean plastic cups (capacity: 15-20 mL). The first bed was wetted with the test sample (RPZ-Na at different concentrations, the same as the *A. cepa* test). Then, the seeds (five for each concentration or sample) were placed on the respective beds, maintaining optimal distance between them. Similarly, the second bed was prepared with small pieces of tissue paper and placed in the distributed seeds in each treatment. The cups were covered with plastic covers and kept in a 12-hour dark-light cycle for 72 hours at 28 ± 1 °C. Each treatment cup containing the bed was wetted with the respective sample or controls every 24 hours. Distilled water and $CuSO_4$ (50 µM) were used as NC and PC, respectively. The shoot and root lengths were measured according to Bhattacharya *et al.* (18). The IC_{50} values were calculated as mentioned above.

Statistical analysis

The analysis of variance (ANOVA) followed by the Tukey post hoc test was determined by using the software GraphPad Prism (version 6.0), considering $p< 0.05$ with a confidence level of 95%. Results are expressed as the mean ± standard error of the mean (SEM).

RESULTS

A. cepa test

Table 1 indicates that RPZ-Na concentration-dependently inhibited root growth (RG) in *A. cepa*. RPZ-Na at 0.4 mM exerted the highest RG inhibition at 72 h. Compared to 24 h, exposure time 72 h showed a time-dependent toxic effect on *A. cepa* roots. The standard $CuSO_4$ exhibited higher toxicity in the test system than the RPZ-Na. RPZ-Na at 0.025 mM showed a negligible toxic effect on the test system compared to the other test concentrations of RPZ-Na. The IC_{50} values calculated at 24, 48, and 72 h were 0.20 ± 0.06, 0.22 ± 0.06, and 0.20 ± 0.08 mM, respectively.

Table 1. Toxic effects of rabeprazole sodium and controls on *Allium cepa* root meristems at different exposure time

Treatments/ at different exposure time		Root length (mm)			Inhibition of root growth (%)			IC_{50} [CI; R^2]		
		24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
NC		53.33 ± 2.91	70.33 ± 2.73	88.33 ± 2.19	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-	-	-
PC		08.02 ± 1.97*	16.38 ± 2.17*	20.39 ± 2.17*	88.71 ± 1.97	76.71 ± 2.17	76.92 ± 2.17	-	-	-
RPZ-Na (mM)	0.025	52.51 ± 3.39	68.28 ± 2.08	87.33 ± 3.58	01.54 ± 3.39	01.13 ± 2.08	01.36 ± 3.58	0.20 ± 0.06 mM [0.14 – 0.31 mM; 0.96]	0.22 ± 0.06 mM [0.15 – 0.33 mM; 0.96]	0.20 ± 0.08 mM [0.13 – 0.31 mM; 0.95]
	0.05	46.23 ± 2.54*	60.67 ± 3.24*	75.56 ± 2.93*	13.31 ± 2.54	13.88 ± 3.24	14.46 ± 2.93			
	0.1	36.13 ± 2.93*	50.36 ± 2.13*	57.33 ± 2.78*	32.25 ± 2.93	28.39 ± 2.13	35.10 ± 2.78			
	0.2	28.67 ± 2.33*	42.33 ± 3.08*	51.12 ± 3.28*	46.24 ± 2.33	39.81 ± 3.08	42.13 ± 3.28			
	0.4	19.33 ± 2.85*	24.67 ± 2.67*	28.67 ± 2.96*	63.75 ± 2.85	64.92 ± 2.67	67.54 ± 2.96			

Values are mean ± standard error of the mean (SEM) (n = 5); ANOVA followed by Tukey post hoc test, considering $p < 0.05$ with a confidence level of 95%; NC: negative control; PC: positive control ($CuSO_4$; 38 µM); RPZ-Na: rabeprazole sodium; IC_{50}: half maximal inhibitory concentration; CI: confidence of interval; R2: coefficient of determination at 95% confidence intervals.

Figure 1 shows the percentage increase in RG profile in the samples and control groups at 48 and 72 h compared to 24 h of exposure time. Both samples and controls did not increase in RG capacity at 72 h inspection of 48 h of exposure time; therefore, this was not shown in the figure. RPZ-Na increased the RG profile at 48 h at 0.025, 0.1, and 0.2 mM, while at 72 h at 0.025 and 0.2 mM. However, compared to 48 h, there was a decrease in the RG profile with these two concentrations of RPZ-Na. The standard CuSO4 also decreased the RG profile at 72 h more than at 48 h of exposure time. RPZ-Na at 0.05 mM and the highest test concentration, 0.4 mM, did not increase RG at 48 and 72 h compared to 24 h as well as 72 h compared to 48 h of exposure time.

A. sativum test

Table 2 shows that RPZ-Na concentration-dependently inhibited root growth (RG) of *A. sativum*. RPZ-Na at 0.4 mM exerted the highest RG inhibition at 24 h. However, at 72 h, it also showed almost similar (64.29 ± 4.12%) inhibition of the RG profile. The standard $CuSO_4$ exerted a more toxic effect on the test system than the RPZ-Na. RPZ-Na at 0.025 mM showed a negligible toxic effect on the test system compared to the other test concentrations of RPZ-Na. RPZ-Na at all concentrations decreased the %inhibition of RG at 48 h compared to 24 h of exposure time, which was then increased at 72 h compared to 48 h of exposure time. Both the standard and RPZ-Na inhibited the RG profile in a time-dependent manner from 24 to 72 and 48 to 72 h exposure time, respectively. The IC_{50} values calculated at 24, 48, and 72 h were 0.21 ± 0.06, 0.36 ± 0.08, and 0.21 ± 0.07 mM, respectively.

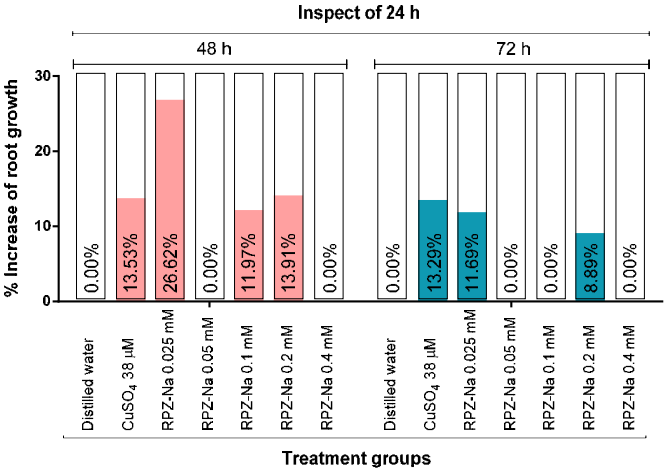

Figure 1. Adaption towards the toxic effects of the test sample and controls on *Allium cepa* compared to 24 h of exposure time [Values are percentage decrease in comparison to the NC (distilled water) group in toxic response in the same treatment group, compared to 24 h of exposure time. Negative values were omitted in the graph. NC: distilled water; PC: positive control ($CuSO_4$)]

Table 2. Toxic effects of rabeprazole sodium and controls on *Allium sativum* root meristems at different exposure time

Treatments		Root length (cm)			%Inhibition of root growth			IC50 [CI; R2]		
		24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
NC		14.20 ± 4.73	44.80 ± 2.78	84.00 ± 4.08	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-	-	-
PC		3.78 ± 1.08*	16.20 ± 2.22*	26.43 ± 2.78*	73.38 ± 1.08*	63.84 ± 2.22*	68.54 ± 2.78*	-	-	-
RPZ-Na (mM)	0.025	13.43 ± 3.76	43.20 ± 3.07	80.20 ± 2.93	05.42 ± 3.76*	03.57 ± 3.07*	04.52 ± 2.93*	0.21 ± 0.06 mM [0.13 – 0.28; 0.96]	0.36 ± 0.08 mM [0.17 – 0.38; 0.91]	0.21 ± 0.07 mM [0.12 – 0.29; 0.95]
	0.05	11.97 ± 1.73*	36.40 ± 2.58*	67.60 ± 4.13*	18.75 ± 2.58*	15.70 ± 1.73*	19.52 ± 4.13*			
	0.1	9.87 ± 2.96*	35.00 ± 1.83*	55.00 ± 3.19*	30.49 ± 2.96*	26.88 ± 1.83*	34.52 ± 3.19*			
	0.2	7.20 ± 2.58*	32.40 ± 4.02*	43.20 ± 2.93*	49.30 ± 2.58*	41.68 ± 4.02*	48.57 ± 2.93*			
	0.4	4.98 ± 2.79*	19.20 ± 3.93*	30.00 ± 4.12*	64.93 ± 2.79*	57.14 ± 3.93*	64.29 ± 4.12*			

Values are mean ± standard error of the mean (SEM) (n = 5); ANOVA followed by Tukey post hoc test, considering $p < 0.05$ with a confidence level of 95%; *$p < 0.05$ when compared to the NC (negative control) group; PC: positive control ($CuSO_4$; 38 µM); RPZ-Na: rabeprazole sodium; IC_{50}: half maximal inhibitory concentration; CI: confidence of interval; R^2: coefficient of determination at 95% confidence intervals.

The percentage increase in RG profile in the test sample and control groups at 48 and 72 h inspection of 24 h exposure time in the *A. sativum* test system (Figure 2). RPZ-Na increased the RG profile at 48 h at all the test concentrations, while at 72 h it was 0.025, 0.2, and 0.4 mM. RPZ-Na showed the highest increase in RG at 0.025 mM (34.13%). However, compared to the 48 h, there is a decrease in the RG profile at these three concentrations of RPZ-Na. The standard $CuSO_4$ also decreased the RG profile more at 72 h than at 48 h compared to 24 h of exposure time. RPZ-Na at 0.05 and 0.1 mM did not increase the RG profile at 72 h compared to 24 and 48 h of exposure time. The sample and controls did not increase RG capacity at 72 h compared to 48 h of exposure time. Therefore, this was not shown in the figure.

C. arietinum test

Both $CuSO_4$ and RPZ-Na decreased the shoot and root lengths in *C. arietinum*. However, their effects were more prominent on the root growth than the shoot growth profile. RPZ-Na significantly ($p < 0.05$) concentration-dependently decreased the shoot and root lengths in *C. arietinum*. At 0.4 mM, RPZ-Na showed the highest inhibition of shoot (57.10 ± 0.58) and root (62.25 ± 0.88) lengths. The standard $CuSO_4$ decreased the shoot and root lengths by 73.99 ± 3.08 and 82.73 ± 2.19%, respectively. The IC_{50} values calculated for the %inhibition of the shoot and root lengths were 0.36 ± 0.12 and 0.13 ± 0.11 mM, respectively (Table 3).

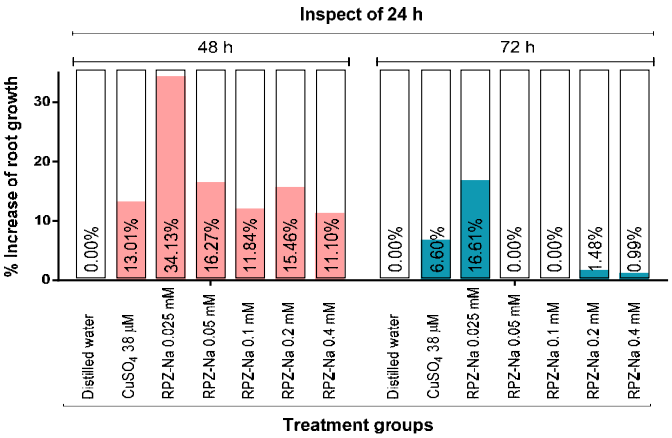

Figure 2. Adaption towards the toxic effects of the test sample and controls on *Allium sativum* compared to 24 h of exposure time [Values are percentage decrease in toxic response in the same group of treatment, compared to 24 h of exposure time. Negative values are omitted in the graph. NC: distilled water; PC: positive control ($CuSO_4$)]

Table 3. Toxic effects on the germination profile of *Cicer arietinum* seedlings by the rabeprazole sodium and controls at 72 h

Treatments		SL (mm)	%ISL	RL(mm)	%IRL	IC_{50} [CI; R^2]	
						ISL	IRL
NC		33.33 ± 4.26	0.00 ± 0.00	83.00 ± 3.22	0.00 ± 0.00	-	-
PC (standard)		8.67 ± 3.08	73.99 ± 3.08	14.33 ± 2.19	82.73 ± 2.19	-	-
RPZ-Na (mM)	0.025	28.00 ± 1.53*	15.10 ± 1.53	66.00 ± 4.58*	20.48 ± 4.58	0.36 ± 0.12 mM [0.07 – 0.39; 0.85]	0.13 ± 0.11 mM [0.06 – 0.26; 0.87]
	0.05	21.67 ± 2.85*	34.98 ± 2.85	52.67 ± 1.23*	36.54 ± 1.23		
	0.1	19.33 ± 0.88*	42.00 ± 0.88	46.54 ± 2.03*	43.93 ± 2.03		
	0.2	17.33 ± 0.88*	48.01 ± 0.88	39.67 ± 3.48 *	52.20 ± 3.48		
	0.4	14.00 ± 0.58*	57.10 ± 0.58	31.33 ± 0.88*	62.25 ± 0.88		

Values are mean ± standard error of the mean (SEM) (n = 5); ANOVA followed by Tukey post hoc test, considering $p< 0.05$ with a confidence level of 95%; NC: negative control; PC: positive control ($CuSO_4$; 50 µM); RPZ-Na: rabeprazole sodium; SL: shoot length; RL: root length; ISL: inhibition of shoot length; IRL: inhibition of root length; IC_{50}: half maximal inhibitory concentration; CI: confidence of interval; R^2: coefficient of determination at 95% confidence intervals.

DISCUSSION

Exploration of drugs or chemicals toxicity towards living organisms is an important step before the industrial and commercial phases of any new drugs and/or chemicals. *In vivo* models consisting of experimental animals and/or their derived cells and tissues are expensive and time-consuming for toxicity assessment. Furthermore, ethical considerations about the handling and sacrifice of animals are other important issues.

Besides *A. cepa,* other species such as *Vicia faba, Tipula paludosa, Pisum sativum, Hordeum vulgare,* and *Crepis capillaris* are also used for toxicological analyses. However, the *A. cepa* test is popularly used to determine toxicity in the laboratory due to its storage facility and availability (20). It is a rapid, precise, and cost-effective plant-based eukaryotic test model. It is well correlated with the higher eukaryotic test models (13). *A. sativum* might also be an economical test model, as it requires only garlic cloves for this assay. Each bulb of garlic produces multiple cloves. Both of these test systems do not require aseptic techniques. Therefore, these assays may complement the 3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyltetrazolium bromide (MTT) assay in toxicology. Thus, the findings from this kind of toxicological study can be applied to animal-based toxicity studies (20). For example, the popularly used PPI omeprazole (OME) was seen to exert concentration-dependent (10, 20, and 40 µg/mL) toxic effects on the *A. cepa* test system (21). In another study, Braga et al. (22) treated Swiss mice with 10, 20, and 40 mg/kg for 14 days and found that OME dose-dependently exerted toxic effects on the stomach, bone marrow, and peripheral blood lymphocytes. Similarly, a clinical study reports that 152 patients using OME at 20, 30, and 40 mg/kg doses for a long time experienced serious toxic effects on stomach cells (23). The root length is an important parameter in *A. cepa* and *A. sativum,* while the shoot length is in the *C. arietinum* test system. These reflect the toxicity of any toxic substance capable of serving as a receptive external signal for steady internal cellular events (18, 21). The toxic substances can accumulate in the roots, resulting in chromosomal aberrations (e.g., C-mitosis, chromosomal bridges, chromosomal tack, and micronuclei formation), inhibiting root growth in *A. cepa* (24). The accumulation of toxicants in the root meristems substantially impairs the microtubule arrangements, thus inhibiting the root growth profiles of *A. cepa* and *A. sativum*. Both toxic and cytotoxic effects of a toxic substance are related to the elongation of the cell cycle in the differentiation phase (25), apical meristematic activity (26), and inhibition of protein synthesis in *A. cepa* meristems (27).

Copper (Cu) accumulates in root test systems (e.g., *A. cepa, A. sativum*) and can inhibit the root growth profile because of chromosomal aberrations (e.g., C-mitosis, the chromosomal bridges, the chromosomal tack, and the micronuclei formation) (24). Generally, Cu accumulated at the root meristems

impairs the microtubule arrangements in these test systems. Therefore, the toxic effects on *A. cepa* and *A. sativum* may be due to an inhibition in root growth, possibly through elongation of the cell cycle during cell differentiation (25), apical meristematic activity (Webster and Macleod 1996), and inhibition of cellular protein synthesis (27).

Cu accumulated more easily at the root rather than at the shoot in *Oenothera glazioviana*. While $CuSO_4$ at 50 µM in a 72-hour exposure time inhibited shoot and root growth with a considerable increase in lipid peroxidation level in the roots of 28-day-old seedlings (28). In our study, $CuSO_4$ at 50 µM also significantly ($p< 0.05$) reduced the shoot length of *C. arietinum* seedlings compared to the RPZ-Na and NC groups. However, PC and RPZ-Na significantly reduced the root length of *C. arietinum* more than the shoot length.

The standard $CuSO_4$ and the test sample RPZ-Na were found to increase the RG profile in *A. cepa* and *A. sativum* test systems at 48 and 72 h compared to 24 h of exposure time. However, this effect of the standard and test samples was not seen at the 72-hour inspection after 48 hours of exposure time. At 72 h, the % increase in RG profile was reduced compared to the 48-h inspection of the 24-h exposure time. It may be due to their damage-preventive capacity, probably by distressing the adaptive response pathways and/or cellular damage-repairing capacity in these test systems. *A. cepa* shows an adaptive response at low concentrations in the Al^{+3}-induced genotoxicity assay (29). This may be due to its genomic protection capacity at low concentrations, regardless of exposure time (30). Therefore, in this study, the test systems *A. cepa* and *A. sativum* may show such adaptive responses at 48 and 72 h compared to 24 h of exposure time. However, at 72 hours compared to 48 hours of exposure time, the damaging events in these test systems may continue.

Finally, the test systems used in this study showed similar responses toward the standard and test samples, especially when compared to the effects on their RG profiles. The coefficient of determination (R^2) values ranged from 0.85 to 0.96 at 95% confidence intervals, suggesting the toxicity study in these eukaryotic test systems is significant. The test sample and the standard used in this study inhibited the root length of the cloves of *A. sativum* and *C. arietinum* seedlings. These two test systems showed similar responses to the widely used test model, *A. cepa*. Therefore, *A. sativum* and *C. arietinum* test models can be incorporated into the toxicogenetic analysis of various substances in different areas of toxicological research.

CONCLUSIONS

RPZ-Na reduced the average root length on the plant-based eukaryotic test models in a concentration- and exposure-time-dependent manner. It may be due to inhibitory effects on the root meristems of *A. cepa* and *A. sativum*. Moreover, RPZ-Na also decreased the concentration- and time-dependently inhibited root and shoot lengths of *C. arietinum*. Together, RPZ-Na exerted more toxic effects on the test systems at 0.1 to 0.4 mM. These findings highlight the potential risks of RPZ-Na to plant growth and development, particularly at higher concentrations.

LIST OF ABBREVIATIONS

FDA: Food and Drug Administration; **GERD**: Gastro-esophageal reflux disease; **PPI**: Proton pump inhibitor; **RG**: Root growth; **RPZ**: Rabeprazole; **RPZ-Na**: Rabeprazole sodium.

ACKNOWLEDGMENTS

Not declared

COMPETING INTERESTS

The authors declare that they have no competing interests.

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