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RESEARCH ARTICLE

Paracetamol ecotoxicological bioassay using the bioindicators *Lens culinaris* Med. and *Pisum sativum* L

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Abstract

Paracetamol is one of the most widely used drugs worldwide, yet its environmental presence and hazardous impact on non-target organisms could rapidly increase. In this study, the possible cytotoxic effects of paracetamol were evaluated using two bioindicator plants *Lens culinaris* and *Pisum sativum*. Concentrations of 500, 400, 300, 200, 100, 50, 25, 5, 1 mg L^{-1}, and a control (distilled water) were used for a total of 10 treatments, which were subsequently applied on seeds of *Lens culinaris Med*. and *Pisum sativum L.*; after 72 h of exposure, root growth, mitotic index, percentage of chromosomal abnormalities, and the presence of micronucleus were evaluated. The cytotoxic effect of paracetamol on *L. culinaris* and *P. sativum* was demonstrated, reporting the inhibition of root growth, the presence of abnormalities, and a significant micronucleus index at all concentrations used, which shows that this drug has a high degree of toxicity.

Keywords Binucleate cell · Cell abnormalities · Pharmacological compounds · Mitotic inhibition · Micronuclei

Introduction

Paracetamol (PCT) (also known as acetaminophen; 4-acetaminophenol; 4-hydroxyphenyl-acetamide) is one of the most widely used medications in different therapeutic procedures worldwide due to its antipyretic and anti-inflammatory properties (Freo et al. 2021). Its molecular weight is 151.16 g/mol; each molecule consists of a benzene ring substituted by a hydroxyl group and the nitrogen atom of an amide group (Phong et al. 2019; Freo et al. 2021). In 1893, PCT was found in the urine of people who had ingested phenacetin and was isolated as a white, crystalline compound with a bitter taste. Then, in 1899, PCT was identified, and since then it has been considered a metabolite of acetanilide (Freo et al. 2021). Even its high use is due to the uncontrolled availability of the medicament, it is an over the counter and inexpensive product that is also available as an active ingredient in more than 600 preparations (Cameron et al. 2021).

During the COVID-19 pandemic, this drug was one of the most widely used medicines for symptom relief in therapeutic activities prescribed by health professionals (Mostafa et al. 2022; Lapi et al. 2022; O'Keefe et al. 2021; Quispe-Cañari et al. 2021; Cameron et al. 2021; Pandolfi et al. 2022) and as self-medication during the quarantine period (Quispe-Cañari et al. 2021; Faqihi and Sayed 2021), generating an increase in PCT consumption, which led to a shortage of this drug (Spyres et al. 2021; Romano et al. 2021).

Acetaminophen is used for its properties that relieve pain and inflammation; at the same time, it can cause some adverse events such as dose-related hepatocellular necrosis, and it is responsible for almost 500 deaths annually in the USA (Lee 2017). Biotransformation of this drug results in inactive compounds by the formation of sulfates and glucuronide conjugates. Likewise, its diffusion occurs through most body fluids in humans, so it is subsequently excreted by the kidneys, thus recovering between 30 and 55% of the drug in the urine during the first day with the therapeutic dose (Prescott 1980). Only a small route is metabolized by the cytochrome P-450 enzyme system, resulting in a metabolite called N-acetyl-p-benzoquinone imine (NAPQI). Under normal conditions, NAPQI is neutralized by the action of glutathione, but, when concentrations are significantly high,

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the sulfate and glucuronide metabolic pathways become saturated. More PCT is diverted to the oxidative system and consequently, glutathione supplies are depleted. NAPQI can freely react with cell membranes, resulting in the profound production of reactive oxygen species (ROS), causing extensive damage and even cell death (Saeedi et al. 2022; Foufelle and Fromenty 2016).

The different PCT fates upon disposal are well known; their chemical compound or metabolites enter ecosystems via wastewater treatment plants (Reinstadler et al. 2021; Landrigan et al. 2020). Concentrations above 65 ug/L in surface water (Roberts and Thomas 2006) and up to 81 ug/kg in soil (Ashfaq et al. 2017) have been demonstrated over time. This is now a well-established problem, as these molecules can confer potential toxicity to other organisms (Parolini 2020). Since the toxicity of p-aminophenol and p-benzoquinone (products of PCT synthesis) is much higher than that of the drug in question, the potential environmental risk of this drug could increase during the formation of intermediates with a higher molecular weight, because these metabolites can accumulate and reach a maximum concentration (Liang et al. 2016).

Pharmaceuticals represent an important group of emerging pollutants that can be taken up by plants (Rede et al. 2019; Leitão et al. 2021). Badar et al. (2022) conducted an experiment on spinach treated with 50 mg/L, 100 mg/L, and 200 mg/L PCT. After 4 days, the concentrations in the roots had a significant reduction to 2.3 µg/g, 2.6 µg/g, and 3.6 µg/g, respectively. This proves its assimilation by these organisms. Currently, the PCT-mediated impact on the environment is presumed to be due to its toxicity, physicochemical properties, and bioavailability (Meffe et al. 2021). Indeed, considerable data have confirmed the hypothesis that PCT could be a problematic compound for aquatic organisms (Bebianno et al. 2017; Gutiérrez-Noya et al. 2021). Daniel et al. (2019) have shown that exposure to PCT at intermediate concentrations between 20 and 40 ug/L produces significant inhibition of glutathione-S-transferase (GST) in *Daphnia magna* individuals. At the same time, the significant DNA lesions found at the end of exposure (Parolini et al. 2010) may show a possible delay of the genotoxic action of paracetamol that can be confirmed by some bioassays performed (Meffe et al. 2021).

At present, plant species continue to be used as a genetic model to evaluate the toxicity of chemical compounds. Since plants are one of the organisms naturally exposed to different environmental pollutants, it becomes important to use them as eukaryotic models that allow environmental risk assessment for ecotoxicological studies (Qi and Zhang 2020). Pharmaceuticals do not follow the trends observed for common anthropogenic pollutants. Drugs pose new challenges to the environment since they are continuously released in amounts comparable to pesticides, remain biologically active in nature, exert effects at extremely low levels, exhibit lipophilicity, and rapidly cross biological membranes. In addition, they are resistant to standard water treatment procedures and can be effective in a multiplicity of organisms that share pharmacological similarities (Nunes et al. 2014).

Eukaryotic system monitoring relies on multiple highly coordinated network functions of the DNA damage response (Nikitaki et al. 2018). The conserved features highlighted in plants and animals represent a challenging opportunity to develop new research available for monitoring in environmental applications, taking advantage that in recent years the literature has focused on ecotoxicity in the soil–plant system (Chen et al. 2021) since, with this system, different procedures are important for a toxicity assessment and learning such as origin, destination, uptake, detoxification, and metabolism can be better evidenced.

The use of bioindicators with plant seeds over the years has been one of the scientific techniques reported for the determination of cytotoxicity, proving to be an instrument of high sensitivity and easy handling (Mendoza and Salazar 2022; Mercado and Caleño 2021; García-Medina et al. 2020; Azzazy 2020; Leston et al. 2013; Nikitaki et al. 2018; Kummerová et al. 2013). This is how plant species have been used for the identification of cytological changes caused by multiple chemical agents such as *Allium cepa*, *Lens culinaris*, and *Pisum sativum* Salazar and Quintero Caleño (2020), also recommended for their economic and practical efficiency, considering that the increasing production, the use of this drug, its environmental presence, and the hazardous impact on aquatic organisms and other non-target individuals could increase rapidly. This study evaluates the possible cytotoxic effects of paracetamol using *Lens culinaris Med.* and *Pisum sativum L.* The negative impact of this drug is presented, demonstrating a high level of toxicity through the inhibition of root growth, the presence of abnormalities, and a significant micronucleus index in all the concentrations used.

Materials and methods

Toxicity test conditions

Lentil (*L. culinaris*) and pea (*P. sativum*) seeds were used as bioindicators to identify cytological changes. To determine the concentrations of paracetamol suitable for the cytotoxicity assay, an exhaustive review was carried out in PubMed and ScienceDirect databases. Nine treatments were carried out with the following paracetamol concentrations (LAFRANCOL S.A.S): 500, 400, 300, 200, 100, 50, 25, 5, 1 mg/L (Kudrna et al. 2020; Rede et al. 2019; An et al. 2009; Svobodnikova et al. 2020) and a control (distilled water) for a total of 10 treatments that were subsequently applied on the seeds of *L. culinaris* and *P. sativum.* For the preparation

of each concentration of paracetamol, distilled water was used because of its easy solubility of 12.78 mg/mL at 20 °C. Concentrations are not analytically confirmed, as is usually the case in the evaluation of screening level approaches (Hillis et al. 2011).

Continuing with the assay proposed by Salazar et al. (2020), 35 seeds of each of the bioindicator species (*L. culinaris Med.* and *P. sativum L.*) were placed in a 100-mm-diameter Petri dish with 90-mm-diameter filter paper and porosity level equivalent to Whatman® N°3 paper inside. Subsequently, 35 mL of each of the concentrations was added to each Petri dish and sealed with Parafilm® paper, and then placed in the dark for 72 h at a temperature of 28 + / − 2 °C to avoid desiccation (An et al. 2009; Bagheri et al. 2021). It is important to mention that all bioassays were performed in 5 replicates (Salazar et al. 2020).

Root growth

After 72 h of exposure to each of the paracetamol concentrations, the root growth of *L. culinaris* and *P. sativum* was evaluated. The root length of each plant was measured, and those that achieved a length greater than 1 mm were taken into account (Di Salvatore et al. 2008); this procedure was followed with some modifications applied by Salazar et al. (2020). For the determination of root growth, the relative root growth percentage (RGRC) formula was applied (Bosker et al. 2019):

$$\mathrm{RRG}(\%) = \frac{\text{Mean root lenght in concentration}}{\text{Mean root lenght in control}} \times 100$$

The root elongation test of a plant is one of the simplest biomonitoring methods for toxicity assessment of organic and inorganic compounds (Aguiar et al. 2016; Priac et al. 2017; Lyu et al. 2018; Di Salvatore et al. 2008). Seed germination starts with water absorption and culminates when the radicle protrusion is present through the enveloping layers (Wolny et al. 2018).

Cell abnormalities

The *L. culinaris* and *P. sativum* tests were used to determine the rate of abnormalities. The following formula applied in the methodology of Salazar and Maldonado Bayona (2020) was used:

$$\text{Relative abnormality rate } (\%) = \frac{\text{Total number of abnormal cells}}{\text{Total number of cells observed}} \times 100$$

During mitosis, some fragments or complete chromosomes are left outside the nucleus, which are called micronuclei (Kalsbeek and Golsteyn 2017; Luzhna et al. 2013). The micronucleus index (MNI) will be determined by the formula used by Scherer et al. (2019):

$$\mathrm{MNI} = \frac{\text{Total of cells with micronucleus}}{\text{Total of cells observed}} \times 100$$

Mitotic index

After a period of seed growth (after 72 h), root tips of approximately 5-mm size of *L. culinaris* and *P. sativum* were rinsed with tap water and stained for 10 min with the compound aceto-orcein (Causil et al. 2017). For the staining protocol, 6 slides were prepared for each treatment, and 1000 cells per 5 replicates were analyzed; pressure was exerted with a coverslip to make each cell phase visible by scattering the cells (Fiskesjö 1985; Salazar et al. 2019; Salazar and Maldonado Bayona 2020). The mitotic index (MI) was calculated, which allows knowing if there is presence or inhibition of cell division and is defined as the ratio between the number of cells undergoing mitosis and the total number of cells (Datta et al. 2018); for this, the following formula was used:

$$\mathrm{Mitoticindex}(\%) = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

For the interpretation of the results, it was considered that, when there are inhibitory alterations, the MI is lower than the value of the negative control. On the other hand, if the MI is higher, it indicates an increase in cell division due to the chemicals used in the assay (Restrepo et al. 2012).

Experimental design and statistical analysis

A completely randomized block design was performed, consisting of an experiment with 9 treatments of different concentrations of paracetamol plus a control group (distilled water), which had an exposure time of 72 h. An analysis of variance (ANOVA) and Tukey's HSD (honestly significant difference) multiple range test ($P \leq 0.05$) using Statgraphics Centurion version XVII statistical software were used for the statistical analysis of the data obtained from the mitotic index and cell abnormalities.

Results and discussion

Root growth

When *L. culinaris* and *P. sativum* seeds were exposed to hydration, the objective was to stimulate cell growth so that the root meristematic cells would elongate. However, when roots are subjected to chemicals, some variations are possible; hence, the degree of affectation will depend on the

chemical and time of exposure (Salazar et al. 2019). The relative growth of the bioindicator species roots was affected in each treatment, and there was a marked decrease; this occurs because the roots are usually the first tissue exposed, where the toxicant inhibits their extension and proliferation, which ultimately compromises the growth and reproductive capacity of the plant (Talukdar and Talukdar 2014).

The uptake of acetaminophen by plants is highly dependent on the sorption and degradation of pharmaceuticals and the molecular weight of medicines (Bartha et al. 2010; Bagheri et al. 2021; Chuang et al. 2019). Acetaminophen has been detected in leaves, soil, and in higher amounts in radish roots (Li et al. 2018). *Spinaca oleracea* plants treated with 50 mg/L, 100 mg/L, and 200 mg/L acetaminophen accumulated 75 µg/g, 136 µg/g, and 1012 µg/g acetaminophen, respectively, after 4 days in shoot tissues. After 8 days, drug concentrations decreased significantly to 56 µg/g, 73 µg/g, and 396 µg/g/g in the 50 mg/L, 100 mg/L, and 200 mg/L treatments, respectively, indicating that biodegradation of acetaminophen occurs after day 4 (Badar et al. 2022).

Concerning the results presented in Table 1, the statistical analysis showed a marked difference between the concentrations used with respect to the root growth of the control treatment (TT1). In the case of *P. sativum* species, there were no significant differences in the following treatments: TT3, TT4, TT5 (5, 25, 50 mg L^{-1} respectively), TT6, and TT7 (100, 200 mg L^{-1}, respectively). Likewise, between TT8, TT9, and TT10 (300, 400, 500 mg L^{-1}, respectively); while, in *L. culinaris*, no differences were shown between TT2, TT3, TT4 (1, 2, 25 mg L^{-1}, respectively) and neither in TT8, TT9, TT10 (300, 400, 500 mg L^{-1}). Even so, it could be observed that root length decreases according to the increase in paracetamol concentrations, considering that the lowest concentrations conserve higher growth figures than the rest of the treatments; this behavior was reflected in the two species used in this research.

Table 1 Root growth and percentage of relative root growth (RRG)

Paracetamol concentration mg L^{-1}	Root length (cm)		Relative root growth percentage (RRG)	
	P. sativum	*L. culinaris*	*P. sativum*	*L. culinaris*
TT1: control	3.96 ± 0.3a	4.34 ± 0.03a	—	—
TT2: 1	3.32 ± 0.2b	3.71 ± 0.05b	83.3	85.4
TT3: 5	2.88 ± 0.1c	3.6 ± 0.44b	72.7	82.9
TT4: 25	2.4 ± 0.1c	3.48 ± 0.23b	60	80.1
TT5: 50	2.472 ± 0.2c	2.95 ± 0.2c	62	56.4
TT6: 100	1.824 ± 0.2d	2.55 ± 0.07d	45	58.7
TT7: 200	1.326 ± 0.1d	2.09 ± 0.06e	32.8	48.15
TT8: 300	0.828 ± 0.07e	1.7 ± 0.12f	20.2	39.1
TT9: 400	0.7 ± 0.08e	1.61 ± 0.11f	17.6	37
TT10: 500	0.48 ± 0.1e	1.59 ± 0.89f	12.2	36.6

Means with different letters show significant differences according to Tukey ($P \leq 0.05$)

According to the above, in some investigations on the effects of paracetamol on germination and growth of plants exposed to different concentrations of 0.01 and 10,000 ng g-1 (Rede et al. 2019) and 0.1; 1; 5 mg/L (Leitão et al. 2021), no significant effects were found since seed germination acts as a protective barrier from negative effects that may be generated by pharmaceutical contaminants (Rede et al. 2019). Something similar was reported with the use of *Lemna gibba* and *Lemna minor*, two species in which a negligible threat was observed in terms of parameters such as photosynthetic pigments, proline levels, and malondialdehyde (MDA) content (Nunes et al. 2014); this makes the measurement of root growth a parameter that provides greater sensitivity when assessing cytotoxicity. In addition, the concentrations used in the investigations are not severe enough to affect plant growth.

On the contrary, other investigations have reported the similarity of statistically significant results in radicle elongation, which indicate that the rate of root inhibition was directly proportional to the concentration of paracetamol (An et al. 2009), results very similar to those observed in this research. In *Cucumis sativus*, the biomass of leaves and roots decreased significantly after 7 days when the concentration of acetaminophen was higher than 10 mg L^{-1}, assuming that plants may be susceptible to phytotoxicity when exposed to acetaminophen (Sun et al. 2019).

Now, abiotic stress such as the excess of toxicants in the soil causes disturbances in several organelles, generating signals that are integrated to regulate gene expression and other cellular activities (Zhu 2016). The significant response with negative effects of cells upon exposure to paracetamol within 72 h is extremely interesting when combined with the finding of color change in the roots of *L. culinaris* and *P. sativum*, which presented dark coloration at the tip; with this, it is possible to hypothesize that the toxic response provoked by paracetamol is energetically demanding as reported by Jaeschke et al. (2021), since there may be depletion of cellular energy reserves present in metabolically active tissues. It has been suggested that the toxic effects of drugs are mediated by the overproduction of reactive oxygen species (ROS), highly damaging molecules that cause deterioration of several cellular structures: membranes, nucleic acids, proteins, enzymes, and lipids (El-Amier et al. 2019); this has been proven in some plants that possess mechanisms to retain much of the toxicity in the roots (Talukdar and Talukdar 2014), suggesting that the cytotoxic impact of acetaminophen would be mediated by oxidative injury (Kalinec et al. 2014). In the trial with *Hyalella azteca* management, the use of acetaminophen was toxic to the species and induced a significant decrease in the antioxidant system caused by ROS (Gómez-Oliván et al. 2014), which likewise

happened with *P. sativum* treated with cadmium, nickel, and lead (El-Amier et al. 2019), where the MDA content was significantly increased, leading to lipid peroxidation due to ROS generation (El-Amier et al. 2019; Aswani et al. 2019).

Mitotic index and mitotic inhibition

The mitotic index (MI) is a measure to quantitatively assess the cell division of an organism (Kato and Haskins 2023). Therefore, if the MI is low compared to the control treatment, the drug is affecting mitosis. In this case, microscopic analysis of cells reflects a remarkable decrease in the mitotic index (MI) is a measure to quantitatively assess the cell division of an organism (Kato and Haskins 2023). Therefore, if the MI is low compared to the control treatment, the drug is affecting mitosis. In this case, microscopic analysis of cells reflects a remarkable decrease in MI, reaching a maximum of 9 ± 0.7 and 9.8 ± 1.2 at 500 mg L^{-1} treatment in *P. sativum* and *L. culinaris*, respectively. According to several investigations using eukaryotic models as bioindicators, e.g., *Hediste diversicolor* (Nogueira and Nunes 2021), *Hyalella azteca* (Gómez-Oliván et al. 2012), *Daphnia magna* (Daniel et al. 2019), *Crassostrea gigas* (Bebianno et al. 2017), *Dreissena polymorpha* (Parolini et al. 2010), *Gibbula umbilicalis* (Giménez and Nunes 2019), *Phorcus lineatus* (Almeida and Nunes 2019), *Ruditapes philippinarum* (Correia et al. 2016), *Mytilus spp.* (Piedade et al. 2020), *Rhamdia quelen* (Perussolo et al. 2019), *Cyprinus carpio* (Gutiérrez-noya et al. 2020), *Danio rerio* (Xia et al. 2017), *Chlorella sp.* and *Desmodesmus spinosus* (Gomaa et al. 2021), *Brachionus rotundiformis* (Park et al. 2018), *Triticum aestivum* (An et al. 2009), *Cucumis sativus L.* (Sun et al. 2019), *Lactuca sativa* (Leitão et al. 2021), *Brassica juncea L.* Czern (Bartha et al. 2010), *Lemna minor*, and *Lemna gibba* (Nunes et al. 2014), to evaluate the toxicological effect of pharmaceuticals on non-target organisms all agree on cell damage and their integration as do the results presented in mention.

The mitotic change reflected in the cells of *L. culinaris* and *P. sativum* appears to be very similar after 72 h of exposure; it is observed that starting from the use of the 400 and 500 mg L^{-1} concentration (TT4 and TT5, respectively), the mitotic inhibition reaches a percentage of 48.9% and 51% in *P. sativum* and *L. culinaris*, respectively (Table 2), representing a concerning value with respect to the control treatment of almost 50%. The measurement of mitosis inhibition provides valuable information on the relationship between damage and the concentrations used. In this case, *L. culinaris* presented less inhibition with *P. sativum* with 51% when using a concentration of 500 mg L^{-1} (Table 2). The control treatment did not cause inhibition in any of the species studied, inferring that, as the concentration of paracetamol increases, the impact on the cell cycle is more relevant.

Table 2 Mitotic index and percentage of mitosis inhibition

Paracetamol concentration mg L^{-1}	Mitotic index		Mitosis inhibition (%)	
	P. sativum	*L. culinaris*	*P. sativum*	*L. culinaris*
TT1: control	17.6 ± 0.8a	19.2 ± 0.83a	—	—
TT2: 1	15.4 ± 0.9b	17.2 ± 0.44[a],b	12.5	10.4
TT3: 5	14.8 ± 0.4b	16.6 ± 0.5b	15.9	13.5
TT4: 25	13.8 ± 0.5c	15.8 ± 1.6b	21.5	17.7
TT5: 50	12.4 ± 0.6c	13.2 ± 1.3c	29.5	31.25
TT6: 100	11.6 ± 0.9c,d	12.4 ± 1.5c,d	34	35.4
TT7: 200	10.8 ± 0.4d	11.8 ± 0.8c,d,e	38.6	38.6
TT8: 300	10.4 ± 0.8d,e	10.8 ± 0.8d,e	40.1	43.5
TT9: 400	9.2 ± 0.8e	10.2 ± 0.5e	47.7	46.9
TT10: 500	9 ± 0.7e	9.8 ± 1.2e	48.9	51

A similar study reported that increased concentration of lead in lentil roots caused several mitotic abnormalities, exposure to the metal affected the cell cycle of *L. culinaris*, and the results showed that higher concentrations were able to significantly inhibit cell division (Çanl 2018), thus inferring that this plant is one of the most sensitive species to perform cytotoxic investigations such as the one exposed in mention since it demonstrates good performance, and the mitotic process can be appreciated, providing an effective cell assessment under stress effects (Salazar et al. 2020; Salazar and Maldonado Bayona 2020).

Cell abnormality index and micronuclei

Overall, 11 types of abnormalities were found, which demonstrates a significant change in the chromosome structure induced by paracetamol (Table 3, Fig. 2), as for *P. sativum* all the abnormalities found were present at concentrations of 200, 300, 400, and 500 mg L^{-1} (TT7, TT8, TT9, TT10, respectively), while using *L. culinaris*, all abnormalities began to be observed from the concentration of 50 mg L^{-1} (TT5). Thus, with the use of the control treatment, no abnormality was evidenced, while with *L. culinaris* the control treatment reported nuclear lesions (Table 3); this allows us to presumably understand that, similar to the effects observed in the work of Khan et al. (2019), the appearance of these anomalies in the roots could be related to the fact that: first, paracetamol is absorbed by bioindicators, and this once inside the plant interferes with normal cell division and induces cytotoxicity, and second, that paracetamol being a low weight molecule—its affinity to the roots is higher, so its uptake and absorption will be more prominent and the root will be the predominant tissue of accumulation by choice of this agent (Chuang et al. 2019, 2015).

Table 3 Chromosomal abnormalities in *P. sativum* L. and *L. culinaris* Med

Paracetamol concentration mg L^{-1}	*Pisum sativum* L								
	Hyperchromasia	Split chromosome	Micronucleus	Binucleated cells	Cells without a nucleus	Nuclear fragmentation	Nuclear lesion	Sticky metaphase	Anaphase bridging
TT1: control	0a	0a	0a	0a	0a	0a	0a	0a	0a
TT2: 1	8.8 ± 0.8b	0.6 ± 0.9[a],b	1.6 ± 0.7b	0a	0a	0a	6.4 ± 1.3	0a	0a
TT3: 5	13 ± 1b,c	1.2 ± 1[a],b	2.2 ± 0.6b	0a	0a	0a	8.4 ± 2	0a	0a
TT4: 25	16 ± 1c,d	1.2 ± 1[a],b	3.6 ± 1c	0a	2.2Ca	3.0 ± 0.7b	12.2 ± 2.5	3 ± 1b	0a
TT5: 50	16.8 ± 0.8c,d	1.8 ± 0.4,b	5.6 ± 1.2c	0a	8.0 ± 1.7b	5.4 ± 1.1c	16.6 ± 2	4 ± 0.7b,c	0a
TT6: 100	19.2 ± 0.8c,d,e	2.6 ± 0.5c	6.8 ± 0.8d	4.0 ± 0.7b	10.6 ± 1.1b,c	7.0 ± 0.7c	21.4 ± 1.6	4.6 ± 0.5c	0a
TT7: 200	21.8 ± 2e	2.8 ± 0.8c	7.8 ± 0.8d	5.6 ± 0.4b	12.2 ± 1.9c,d	8.6 ± 0.4d	23 ± 2.8	6.8 ± 0.8d	6.4 ± 1b
TT8: 300	26.4 ± 2.4e	3.4 ± 0.5c,d	9.6 ± 1e	7.8 ± 0.8d	13.8 ± 1.7d,e	10.6 ± 0.8e	31 ± 3.3	7.0 ± 0.7d	9.2 ± 0.9c
TT9: 400	41.6 ± 3.4f	3.8 ± 0.8c,d	10.4 ± 0.8e	9.2 ± 1.1d	15.8 ± 1.3e,f	11.4 ± 0.5e	37 ± 1.5	7.4 ± 0.5d	10.6 ± 0.8c,d
TT10: 500	44.8 ± 3.5f	4.6d	11.6 ± 0.5f	11.8 ± 1.e	17.2 ± 0.8f	13.2 ± 0.8f	37.8 ± 1	9.8 ± 0.8e	12.2 ± 1.7d
Paracetamol concentration mg L^{-1}	*Lens. culinaris* Med								
	Split chromosome	Micronucleus	Cells without a nucleus	Lagging chromosome		Anaphase bridging	Sticky metaphase	Irregular anaphase	Nuclear lesion	Binucleated cells
TT1: control	0a	0a	0a	0a		0a	0a	0a	0.2 ± 0.4	0a
TT2: 1	0a	0.8 ± 0.5a,b	0a	0a		0a	4.0 ± 0.8b	0a	8.4 ± 8.4	0a
TT3: 5	0a	1.8 ± 0.4b,c	4.0 ± 1b	0a		0a	5.0 ± 1.2b,c	0a	17.8 ± 1	0a
TT4: 25	3.8 ± 0.4b	3.0 ± 0.7c	7.8 ± 0.9c	0a		0.6 ± 0.35a	5.8 ± 0.8b,c,d	1.2 ± 0.7[a],b	21.6 ± 2	0a
TT5: 50	3.8 ± 1.3b	5.8 ± 0.8d	9.6 ± 1c	1.6 ± 0.3b		2.4 ± 0.4b	6.0 ± 0.7b,c,d	1.4 ± 0.5[a],b	28.2 ± 1	3.6 ± 0.85b
TT6: 100	4.0 ± 0.7b	10 ± 0.9e	12.6 ± 0.8d	2.6 ± 0.5b,c		2.6 ± 0.5b	5.0 ± 1.2,c	2.4 ± 0.6b	31.8 ± 1.4	5.2 ± 0.8b,c
TT7: 200	4.0 ± 0.7b	11.6 ± 0.8e	14.2 ± 0.7d	3.8 ± 0.6c,d		3.4 ± 0.8b,c	7.0 ± 1 cd	2.4 ± 0.8b	40.8 ± 4.1	5.4 ± 1.5c
TT8: 300	5.4 ± 0.5c	16.2 ± 1.6	17.2 ± 0.8e	4.6 ± 0.7d,e,f		3.6 ± 0.5b,c,d	7.8 ± 0.4e	3.8 ± 0.3	47 ± 3.8	7.4 ± 0.8d
TT9: 400	5.6 ± 0.8c	16.6 ± 0.4f	19.4 ± 1.3e,f	5.4 ± 1.2f		4.2 ± 1.4c,d	9.6 ± 0.5e	4.8 ± 0.4	48.0 ± 3.4	9.2 ± 0.4e
TT10: 500	8.4 ± 0.5d	17.6 ± 0.5f	21. 6 ± 2f	5.5 ± 0.9f		5 ± 0.7d	12.2 ± 2.9f	5.4 ± 1	51.2 ± 5	9.4 ± 1.1e

Means with different letters show significant differences according to Tukey ($P \leq 0.05$).

For the interpretation of the obtained results, fundamentally, it is necessary to highlight that chromosomes are thread-like structures made of proteins and DNA organized in a compact form to allow the precise transmission of genetic material to daughter cells in mitosis; this error-free process depends on the precise attachment of chromosomes to spindle microtubules (Liu et al. 2020; Sharp. 2002), as can be seen in Fig. 1, the progression of a normal cell cycle. In the final mitotic phase, extensive remodeling ensures that the separated chromosomes are surrounded by a nuclear envelope and that a single nucleus is formed in each daughter cell. However, cellular atypia may exist in the mitotic process (Kwon et al. 2020), and one of the reasons that may disrupt this cycle is attributed to the abiotic stress to which plants are exposed by their frequent confrontation with environmental changes (Qi and Zhang 2020).

The most frequent abnormality observed in this assay was the presence of micronuclei (Fig. 2C, Table 3), and conversely, anaphase bridges (Fig. 2I, Table 3) were the least frequent abnormality. Micronuclei form when a chromosome or an acentric fragment fails to properly bind to the mitotic spindle, is delayed during anaphase, and is not incorporated into one of the two primary nuclei; nuclear envelope disruption in micronuclei is often irreversible and is associated with DNA damage of unknown cause (Maciejowski and Hatch 2020; Luzhna et al. 2013; Sommer et al. 2020). The significance of this finding is that micronuclei are not transient cellular events and that on the contrary, they are associated with chromosomal unstable consequences that may contribute to tumor formation (Blackford and Stucki 2020). Furthermore, the report of this cellular aberration allows for the inference that substantial DNA damage may be associated with oxidative stress pathways and effects that could drive a tumorigenic response.

However, these data must be examined in the context of paracetamol concentrations, mode of action considerations, and the metabolism that occurs in each species for detoxification; what is relevant is that if there is DNA fragmentation, it could result in a cancer cell. In other words, the causes of tumor growth in plant cells include the acquisition of meristematic characteristics by differentiated cells (Dodueva et al. 2020), i.e., it is important to have control of plant cell proliferation to specify normal development.

According to Table 3, it is likely that there are some hypotheses to explain why *P. sativum* showed less frequency in the abnormalities in common compared to *L. culinaris*, possibly due to a lower sensitivity to the toxicant or that each species has a different metabolism that allows resistance to pharmacological residues with greater tolerance than others. In general terms, a trend was observed in which treatment 10 (500 mg L^{-1}) caused the highest frequency of abnormalities. In the same way, the relative abnormality rate where the micronucleus index and the relative percentage of abnormality are evaluated together (Table 4) correlates with the results obtained in Table 3, showing that the action of paracetamol causes irreversible

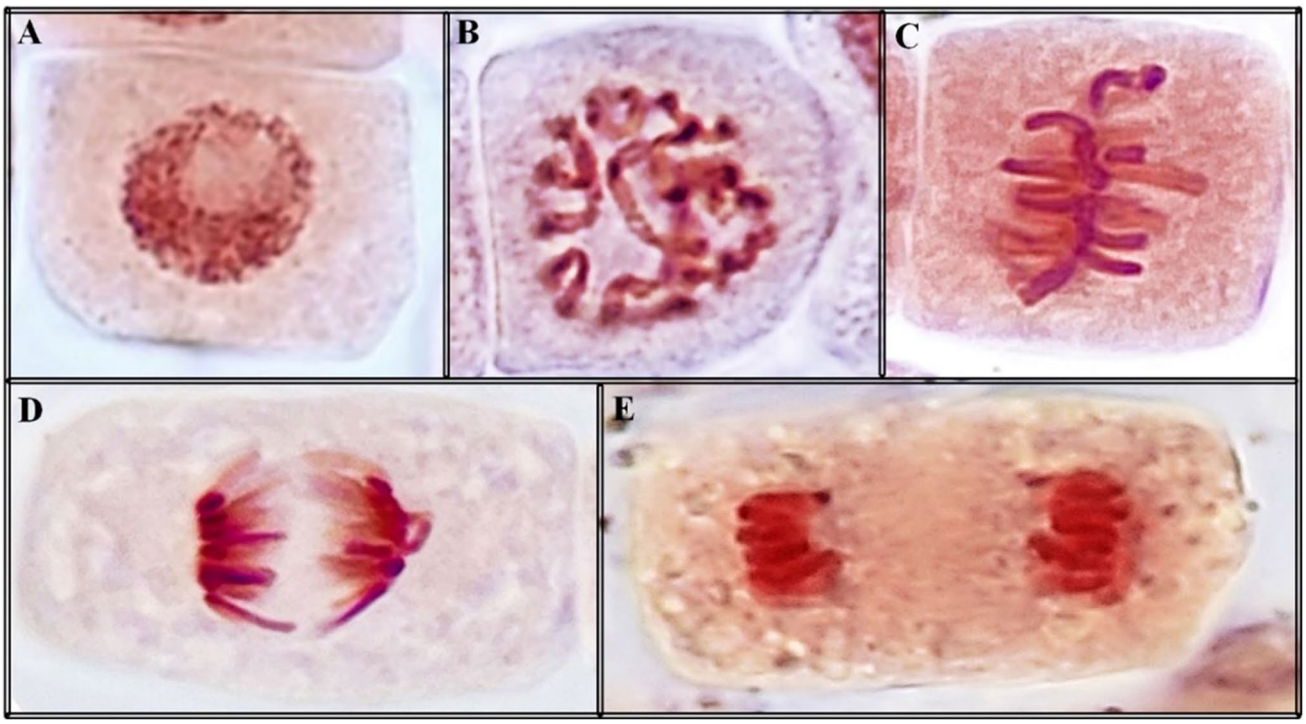

Fig. 1 Phases of the cell cycle in *P. sativum*: **A** interphase; **B** prophase; **C** metaphase; **D** anaphase; **E** telophase

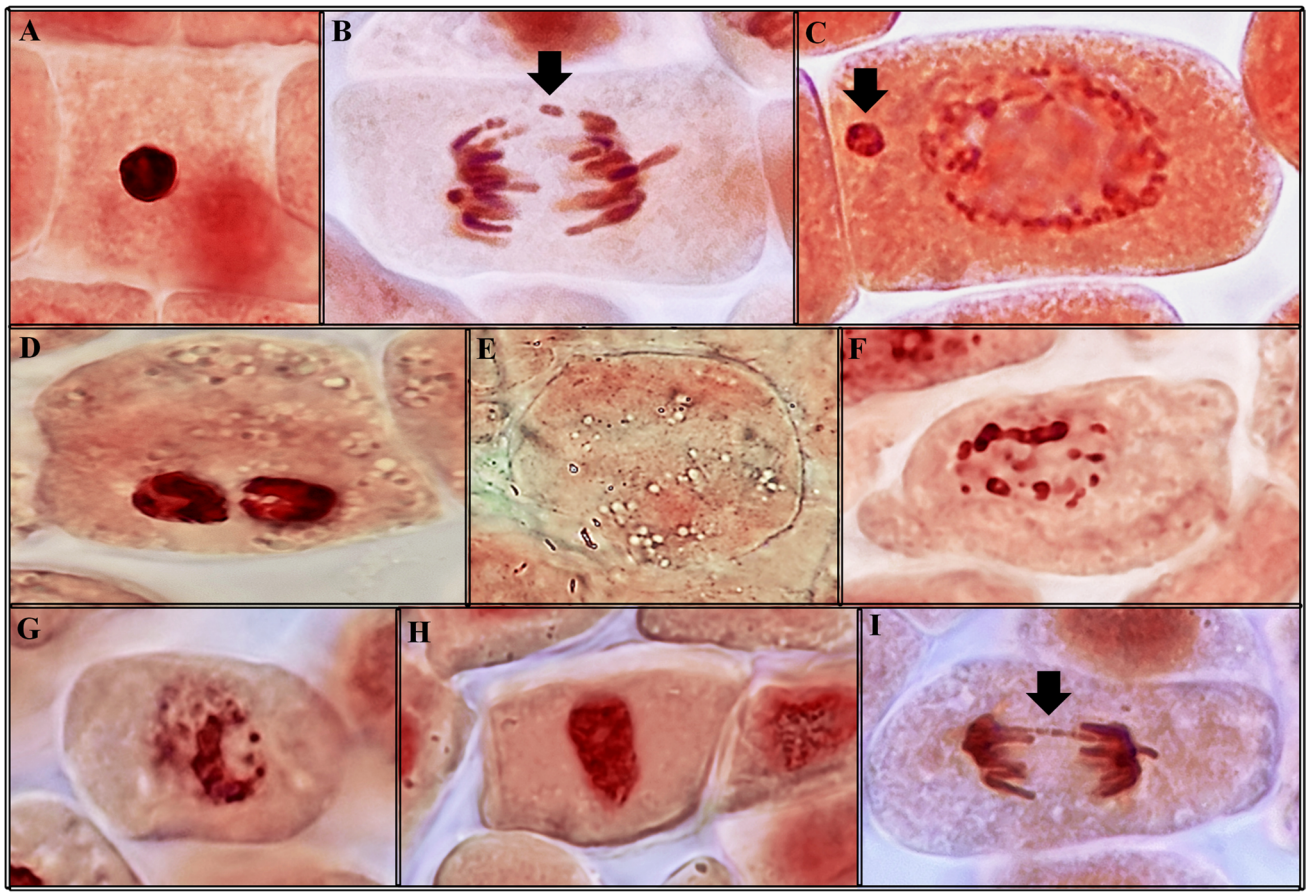

Fig. 2 Chromosomal abnormalities found in the mitotic process of *P. sativum* root: **A** hyperchromasia; **B** split chromosomes; **C** micronucleus; **D** binucleate cell; **E** cells without a nucleus; **F** cell fragmentation; **G** nuclear lesion; **H** sticky metaphase; **I** anaphase bridge

DNA damage, and this is an important finding for plant development, since, as other authors have discussed, the probable mechanisms leading to DNA and/or chromosome damage are invariably associated with cell lethal processes and carcinogenesis (Kirkland et al. 2021). In this sense, the use of two plant biomarkers in this research represents higher reliability in the results, because it allows a statistical comparison as discussed in detail above. On the other hand, all paracetamol concentrations produced anomalies in common with values similar to each other, which allows inferring that paracetamol represents a toxicological risk to the environment itself.

Conclusions

The toxicity of paracetamol was evaluated in two plant species *L. culinaris* Med. and *P. sativum* L., demonstrating cytotoxic effects in the roots of the used bioindicators. Inhibition of root growth, the presence of abnormalities in the mitotic process, and a concerning micronucleus index were observed at all used concentrations. These data pose a problem for plant metabolism, and the results are critical, demonstrating that this medication has a high degree of toxicity. This study suggests that *L. culinaris Med.* and

Table 4 Relative abnormality rate: micronucleus index and relative percentage of abnormality

Paracetamol concentration mg L^{-1}	Micronuclei index (%)		Relative rate of abnormality (%)	
	P. sativum	*L. culinaris*	*P. sativum*	*L. culinaris*
TT1: control	0a	0a	0	0.02
TT2: 1	0.16 ± 00.7b	0.08 ± 0.05a,b	1.4	1.32
TT3: 5	0.2 ± 00.6b	0.18 ± 0.04b,c	2.48	2.86
TT4: 25	0.36 ± 0.1c	0.3 ± 0.07c	5.82	4.38
TT5: 50	0.56 ± 0.12c	0.58 ± 0.08d	7.62	6.2
TT6: 100	0.8 ± 00.8d	1 ± 0.09e	7.62	7.6
TT7: 200	0.78 ± 00.8d	1.16 ± 0.08e	9.5	9.26
TT8: 300	0.96 ± 0.1e	1.62 ± 0.16	11.78	11.3
TT9: 400	1 ± 0.08e	1.7 ± 0.04f	14.7	12.2
TT10: 500	1.2 ± 00.5f	1.8 ± 0.05f	16.3	13.6

P. sativum L can be used as biological models to apply ecotoxicological bioassays. However, despite the use of a wide range of PCT concentrations, it is important to analytically validate the concentrations and characterize the active metabolites of the pharmaceutical compound.

Author contribution Seir Antonio Salazar Mercado: methodology, writing, and original draft preparation. Jesús David Quintero Caleño: supervision, conceptualization, investigation, and data curation.

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Data availability The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

Declarations

Ethical approval This section is "not applicable" for this study as the study does not involve any human participants nor their data or biological material.

Consent to participate Written informed consent was obtained from individual or guardian participants.

Consent for publication This section is "not applicable" for this study as the manuscript did not include any data from individuals.

Competing interests The authors declare no competing interests.

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