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Toxicogenetic study of omeprazole and the modulatory effects of retinol palmitate and ascorbic acid on *Allium cepa*

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Abstract

Omeprazole (OME) is a proton pump inhibitor used for treatment of various gastric and intestinal disease; however, studies on its effects on the genetic materials are still restricted. The present study aimed to evaluate possible toxicogenic effects of OME in *Allium cepa* meristems with the application of cytogenetic biomarkers for DNA damage, mutagenic, toxic and cytotoxic effects. Additionally, retinol palmitate (RP) and ascorbic acid (AA) were also co-treated with OME to evaluate possible modulatory effects of OME-induced cytogenetic damages. OME was tested at 10, 20 and 40 µg/mL, while RP and AA at 55 µg/mL and 352.2 µg/mL, respectively. Copper sulfate (0.6 µg/mL) and dechlorinated water were used as positive control and negative control, respectively. The results suggest that OME induced genotoxicity and mutagenicity in *A. cepa* at all tested concentrations. It was noted that cotreatment of OME with the antioxidant vitamins RP and/or AA significantly ($p < 0.05$) inhibited and/or modulated all toxicogenic damages induced by OME. These observations demonstrate their antigenotoxic, antimutagenic, antitoxic and anticitotoxic effects in *A. cepa*. This study indicates that application of antioxidants may be useful tools to overcome OME-induced toxic effects.

Keywords: Antioxidants; omeprazole; vitamins; *Allium cepa*; cytogenotoxicity.

1. Introduction

Omeprazole (OME) is used for the treatment of gastrointestinal disorders such as dyspepsia, peptic ulcer, gastroesophageal reflux or *Helicobacter pylori* infection (Kosma *et al.*, 2016; Seoane *et al.*, 2017). It is a proton pump inhibitor (PPI) (Yanagihara *et al.*, 2015), that acts through irreversible blocking of the terminal phase of gastric acid secretion in the gut by inhibiting the H^+/K^+ ATPase proton pump in parietal cells. This in turn decreases the amount of acid in the stomach (Paroni Sterbini *et al.*, 2016; Seoane *et al.*, 2017).

Several studies reported that prolong use of OME may cause some abnormalities in the gastric mucosa including parietal cell hyperplasia, dilatation of the canaliculi in the fundus, body and antrum of the stomach, and projection of cytoplasmic protrusions into the lumen of the canaliculi (Kurman, 2013). It is also important to emphasize that at doses of 20 to 40 mg/day, OME can inhibit up to 90% of the 24-hour acid secretion in most patients, resulting in achlorhydria among many patients. This condition long term can lead to gastric cancer (Melbourne, 2013; Lima and Filho, 2014).

OME can also inhibit the gastric acid pump in the parietal cell when it is completely metabolized in the liver releasing sulfone, sulfite and hydroxy-OME, compounds that can generate oxidative damage and induce damage to the genetic materials (Brambilla *et al.*, 2009; Downes and Foster, 2015). Thus, toxicogenic studies indicating primary damages that may progress to the stages of carcinogenesis that are necessary for the identification of potentially mutagenic chemical agents and to assess risks of genetic instability (Zeiger *et al.*, 2015), including drugs (Lee *et al.*, 2014). Kohler (Kohler *et al.*, 2010) indicated that toxic effectets mediated by chronic OME uses could be minimized using antioxidant therapy. Herein, we planned to evaluate efficacy of two compounds namely retinol palmitate (RP) and ascorbic acid (AA) in preventing the toxicity caused by OME treatment. Both of these

compounds are known antioxidant capable of neutralizing free radicals and oxidative stress (Banala and Karnati, 2015; Shao *et al.*, 2016; Asaikkutti *et al.*, 2016).

Several tests can be used for the evaluation of genotoxic and mutagenic effects, among which *Allium cepa* test is an important test system for the evaluation of cytotoxotoxic effects of various substances that may affect genetic materials (Özkara *et al.*, 2015). The results are correlated with the responses obtained from the tests with mammalian systems (Moura *et al.*, 2016). The comet assay used to detect DNA damage can be applied in root meristems of *A. cepa* for the evaluation of genotoxic effects of varieties of substances (Tu¨Rkog˘Lu 2012; Liman, 2013). We, therefore, set out to evaluate the toxicogenic effects of OME on *A. cepa* root meristems with the analysis of cytogenetic biomarkers of DNA damage by the application of the comet test (alkaline version) along with its toxic/cytotoxic and mutagenic effects of the micronucleous (MN) test, as well as the structural alterations of chromosomes in the same test system. Beside this the possible effects of RP and/or AA on OME-induced cytogenetic damage on this test system were also evaluated.

2. Materials and methods

2.1 Source of A. cepa

Fresh and medium size onions (*A. cepa*) were purchased from the local market in Piaui, Brazil. The study was carried out at the Laboratory of Research in Toxicological Genetics, Federal University of Piauí (UFPI), Teresina / PI - Brazil.

2.2 Reagents and chemicals

OME was purchased from a conventional pharmacy, a production of the Medley Indústria Farmacêutica Ltd. and solubilized in distilled water to make the final concentrations of 10, 20 and 40 μg/mL. The usual OME dosage of adult human being ranges from 60 mg to

maximum of 360 mg/day (maximum tolerated dose). This led the authors to select a OME concentration range of 10 to 40 µg/mL because this would be more meaningful in extrapolating the results to human. Because the studies involving the use of OME in plant systems are limited, the dose of OME was selected by applying various concentrations and subsequent analysis. Similar, even higher, concentration of OME was used for physiological studies in tomato plant (Rouphael *et al.*, 2018). Copper sulphate was purchased from the Sigma-Aldrich (St. Louis, MO, USA) and diluted in distilled water to attain at the final concentration of 0.6 µg/mL. Ascorbic acid (AA) and retinol palmitate (RP) were also obtained from the Sigma-Aldrich (St. Louis, MO, USA) and solubilized in phosphate buffer (pH 6.8) and in 0.9% saline plus 10% DMSO to the final concentrations of 35.2 µg/mL and 55 µg/mL, respectively.

2.3 Comet assay with A. cepa

The test was performed with onions (five in each group) according to the method described by Liman (2013). After treatment with carnoy solution, the meristematic regions of the roots were cut into small pieces and treated with 0.4 M Tris-HCl (pH 7.5) overnight to obtain the nuclear extract. Then, by using 1% low melting point agarose (LMPA: 35 °C), the nuclear extract was spread on the previously prepared slides (2.25 % normal melting point agarose (NMPA: 50 °C) in phosphate buffered saline (PBS; pH: 7.4). The slides were then dried in an oven at 37 °C for 1.5 h and the single gel electrophoresis was performed at 25 V, 300 mA for 15 min. The slides were dried as above and then fixed in carnoy solution for 10 min. After three baths in distilled water, the slides were stained and followed by the photomicroscopic observation with magnification of 400X. The genotoxic profile was determined based on the level of DNA damage as described earlier (Paz *et al.*, 2018). The Index of Damage (ID) was obtained by evaluating the tail type, classified from 0 to 4 (50

cells per slide in duplicate) in an optical microscope with the magnification of 1000X. Intact nuclei appear round (Class 0 - no damage), while in the damaged cells, the DNA migrates from the nucleus towards the anode during the electrophoresis, showing a "tail" of sedimented fragments, like a comet: Class 1 (minimum damage) to 4 (maximum damage). Frequency of Damage (FD) was calculated by subtracting cells with zero damage from 100, that is, based on the number of cells with damage vs those without damage. Mitotic Index (MI) is a measure of the proliferation status of a cell population. It is the ratio between the number of cells in a population undergoing mitosis to the total number of cells in a population. It is determined by analyzing micrographs and counting the relative number of mitotic cells versus non-dividing cells. It is noteworthy to mention that except microscopic observation; the entire operation was performed in the dark.

2.4 A. cepa test

The *A. cepa* test was performed according to procedure demonstrated by Islam and coworkers (Islam *et al.* 2017). After purchasing, the onoins were washed with running tap water; removed the old and dried roots, outer membrane, and central parenchyma of the budding crown (making a small circular incision) to facilitate the absorption of the solutions and the uniformity of budding and root growth. After this process, the onions were exposed to each concentration of OME and/or RP and/or AA, and controls and were held for 48 h in the dark at room temperature. After the incubation, the roots were removed and fixed in Carnoy solution (methanol and acetic acid) for 24 h; the roots not evaluated immediately were stored in 70 % ethanol for later analysis in the alkaline comet assay (as previously discussed). For the preparation of the slides, the roots were washed with distilled water (3 baths of 5 min each) to remove the fix and hydrolyzed with 1 N HCl at 60 °C for 11 min. Next, the roots were removed and washed with distilled water followed by drying at room

temperature and transferring to amber colored vials containing Schiff's reagent. Then roots were treated with Schiff's reagent for 2 h followed by washing in running water until complete removal of the dye. The roots were then placed on a slide where the meristematic region was separated, placing a drop of acetic carmine 2% on top and covering with coverslips. The photomicrography was done with an optical microscope at 1000X magnification.

2.5 Stastical analysis

Results are presented as mean ± standard deviation (SD). The data were analyzed by means of analysis of variance (ANOVA) followed by Tukey, Dunnet and Bonferroni's tests by using GraphPad Prism (version 6.0), considering $p < 0.05$ with a confidence level of 95%.

3. Results

OME induced damage to the DNA of *A. cepa* root meristem cells at all concentrations tested by increasing the index (ID) and frequency of damage (FD) in comparisn to the negative control (NC) group. However, RP and/or AA co-treatment, as well as the combination of these two vitamins inhibited the damaging effects of OME 10 μg/mL along with the modulation of 20 μg/mL and 40 μg/mL mM OME-induced damage effects in the test system (**Table 1**).

[Table 1]

The modulation of OME-induced genotoxic damage of RP was 18.4 to 27.9%, whereas that of AA was from 13.2 to 25.2%. However, the association of these two vitamins (RP + AA) increased the modulation from 26.7 to 43.7% (**Figure 1**).

[Figure 1]

The genotoxic damage could not be repaired because several structural changes in chromosomes in meristems of *A. cepa* were observed in anaphases, such as micronuclei (MN), bridges, loose chromosomes, and in the delay, are the biomarkers of mutagenicity. The photomicrograph profile of roots meristem cells shown in **Figure 2** suggests the formation of MN (A), breaks (B) or loss of chromosomes (C and F) observed in the concentration of OME 40 μg/mL, while D and E indicating chromosomal delay separation.

[Figure 2]

Mutagenicity of OME and the modulatory effects of RP and/or AA in *A. cepa* was evaluated in terms of chromosomal alterations (CA) **(Figure 3A)**. Results shows that OME caused dose-dependent (10 to 40 μg/mL) increase in CAs. These observations are indicative of the increased number of structural changes in chromosomes during the cell division process in *A. cepa* meristems especially delayed chromosomal separation. Mitotic defects are characterized by several potential outcomes including failed alignment of chromosomes leading to mitotic arrest/delay enforced by the spindle checkpoint. Several such observations are documented in the form of CAs. The co-treatment of *A. cepa* meristems with OME and RP and AA individually, as well as the combination of OME with RP and AA, showed significant dicrease in CAs (close to NC); howevere, the dose-dependence was not clear. Similarly, the antimutagenic activities of RP and/or AA with OME were observed in *A. cepa* meristems (**Figure 3B**). Results show that the co-treatment of OME and RP and AA individually, as well as the combination of OME with RP and AA, showed significant

169 dicrease in mutagenic activity of OME. The percentage ratio for the inhibition of mutagenic
170 events by RP and /or AA varied from 64 to 74.5% as compared to OME.
171
172 **[Figure 3]**
173
174 OME induced toxicity at the two highest concentrations tested (20 and 40 mg/mL) in
175 *A. cepa* by inhibiting of the root growth (a macroscopic toxicity biomarker). However, in the
176 co-treatment with RP and/or AA, these toxic damages were totally modulated, especially at
177 20 µg/mL concentration of OME. RP inhibited about 47 to 50% toxicity while AA by 15 to
178 44%. However, their association (RP + AA) modulated the OME induced toxic effects by 43
179 to 65% (**Figure 4**).
180
181 **[Figure 4]**
182
183 The cytotoxicity in the *A. cepa* is evaluated by means of the microscopic cell count
184 parameter in the cell division phases. OME induced cytotoxicity only at the highest tested
185 dose (40 µg/mL) (**Figure 5A**). However, the cytotoxic effect of OME was modulated by the
186 RP and/or AA within the range of 16 to 28.8% (**Figure 5B**).
187
188 **[Figure 5]**
189
190 **4. Discussion**
191 Studies of DNA damage at the chromosome level are needed in toxicogenetic
192 studies (Sharma *et. al.*, 2015). In our present study, OME induced genotoxicity by increasing
193 ID and FD in a concentration-dependent manner in *A. cepa* meristematic cells, while

mutagenicity by changing in chromosoms. Moreover, OME also induced toxicity by inhibiting the root growth at the two highest concentrations, while cytotoxicity with highest concentration. OME can alter the bacterial flora of the gastrointestinal tract leading to malabsorption, enteric infections and cause acute or chronic lesions to the cells of the gastric gland, by the compensatory effect, in response to the decreasing of acid with thrombocytopenia, acute interstitial nephritis, nephrotoxicity and hepatotoxicity, anaphylactic reactions, gynecomastia and impotence (Thomson *et al.*, 2010).

In addition to these adverse effects, OME can cause destruction of gastric gland and persistent hypergastrinemia, better to be termed as 'atrophic gastritis' due to OME monotherapy in the context of *H. pylori* infection, has been associated with an increased risk of mucosal dysplasia and gastric cancers (Kohler *et al.*, 2010; Arai and Gallerani, 2011). OME monotherapy, in the context of *H. pylori* infection, increases the risks for mucosal dysplasia and gastric cancer due to the destruction of the gastric glands and persistent hypergastrinemia, denomination for atrophic gastritis (Kohler *et al.*, 2010).

OME can also induce hiccups or achlorhydria leading to the formation of N-nitosamines, which alter the genetic materials due to nuclear abnormalities such as MN, picnoses and karyorrhexis (Novotna *et al*., 2014). It is evident that OME as well as lansoprazole, pantoprazole, phenolphthalein, rabeprazole and sulfasalazine can cause chromosomal damage (Brambilla *et al.*, 2009), as observed in the present study of the formation of MN, bridges, loose chromosomes and delays. Genotoxic damage is indicative of mutagenic effects and is characterized by agents that cause genetic instability, including chromosomal changes, thus the toxic effects (Adeyemo and Farinmade, 2013).

From this study, it is clear that OME induced genetic instability (GI) as it caused damage to DNA and the nuclear abnormalities of chromosomes in *A. cepa*. GI is one of the markers for cancer (Palmeira *et al.*, 2011) and its monitoring is important in therapeutics

especially by changes in chromosomes (Ferguson *et al.*, 2015). Cell division processes often show mistakes which generate changes in chromosome content. Chromosomal segregation errors may produce CA like fragments of whole chromosomes and may lead to like aneuploid or polyploid progeny cells. A major consequence of CA is change in the relative dosage of products from genes located on the missegregated chromosomes (Potapova and Gorbsky, 2017). Chromosomal aberrations may cause mutations and initiate the process of carcinogenesis (Burrell and Swanton, 2014) and the tumor progression (Asatryan and Komarova, 2016) through multiple pathways including telomer damage, chromosome amplification, epigenetic modifications, DNA damage (Ferguson *et al.*, 2015) and changes in proteins such as DNA polymerase and cofactors and breaks of ribbons (Deshmukh *et al.*, 2016).

Another consequence of GIs are the risks of cytotoxicity (by apoptosis), with the consequent of inhibition of the mitotic index (MI). Substances that are cytotoxic may inhibit the growth of vegetative organs in some plants (Yıldız *et al.*, 2009). For example, *A. cepa* (Herrero *et al.*, 2012), as observed in the OME studies at the highest concentration in *A. cepa*, indicates that OME at this concentration may have a mitodepressive action (Sharma and Vig, 2012), characterized by the substances that interfere in the normal cell cycle with decreasing in the number of cells. MI can reliably identify the presence of cytotoxic effects of a substance. A 50% reduction in MI when compared to NC is a limiting value: <50% reduction induces a sub-lethal effect and <22% causes a lethal effect (Mesi and Kopliku, 2013). A possible OME-mediated cytogenetic events has been shown in **Figure 6**.

[Figure 6]

Studies have reported that OME increases apoptosis rates in the treatment of cells together with paclitaxel in chemoresistant cells (HeyA8-MDR, SKOV3-TR) and in clear cell carcinoma (ES-2, RMG-1) (Lee *et al.*, 2015). In human gastric cancer cells (HGC-27), OME selectively inhibited cell proliferation (Zhang *et al.*, 2013). In colon carcinoma in a xenographic model, and in colon cells, with increased secretion of gastrin, OME can increase expression of the X-1 immediate response gene (*IEX-1*), a stress-sensitive gene (Müerköster *et al.*, 2008). The apoptotic properties of OME were also suggested by the reduction of Bcl-2 (Al-Wajeeh *et al.*, 2017), Bcl-xL and survivin (Patlolla *et al.*, 2012), another antiapoptotic protein (Müerköster *et al.*, 2008) and p21 which plays an essential role in stopping the cell cycle after DNA damage (Patlolla *et al.*, 2012).

From the above discussion, several mechanisms may be suggested for the genotoxic, mutagenic, toxic and mitogenic effects of OME, however, studies show that OME can amplify the oxidative stress inherent to gastritis, causing lesions in the gastric mucosa (Kohler *et al.*, 2010). These gastric lesions are also characteristic of chronic atrophic gastritis, precancerous ulcers due to an increasing of oxidative stress in gastric pathologies associated with *H. pylori* (Wadhwa *et al.*, 2013). But there are reports that the drugs may contribute to the elevation of the levels of oxidative stress (Herbet *et al.*, 2016) and that supplementation with these antioxidants ensures the equilibrium of reactive oxygen species (ROS) with the modulation of oxidative stress (Murphy-Marshman *et al.*, 2017).

Thus, OME may cause DNA damage, possibly by unrepaired, while mutagenicity by changing the chromosomal structure observed in anaphases and cytotoxicity by interfering cell division. However, these toxicogenic changes were inhibited and/or modulated by the action of vitamins (RP and/or AA), demonstrating that these vitamins have antigenotoxic, antimutagenic, antitoxic and anti-toxic effects. In order to minimize these changes, there may

induce equilibrium mechanisms between the antioxidant and pro-oxidant effects and are able to neutralize the ROS (Portantiolo *et al.*, 2014).

The RP has ROS neutralizing capacity as it reduces oxidative and nitrosative effects (Moraes *et al.*, 2016), decreases lipid peroxidation, stimulates antioxidant enzymes (Cha *et al.*, 2016), and it acts on the cell cycle (Shao *et al.*, 2016), mitochondrial dysfunction and cellular apoptosis (de Oliveira, 2015). Studies in clinical trials have shown that retinoids are active in the reduction of some primary cancers, such as tumors of the digestive tract, lung and hepatocellular carcinomas (Lee *et al.*, 2010). Thus, despite many controversies, vitamin A precursors such as retinol, RP and carotenoids may be applied in cancer therapies (Felisbino *et al.*, 2014; Rahal *et al.*, 2014), due to its antiproliferative, antioxidant, pro-apoptotic and cell differentiation induction effects (Persaud *et al.*, 2016)

Like RP, AA can interact with free radicals, sequester them and prevent oxidation and damage to DNA, as well as being able to modulate the enzymatic system that generates free radicals and increases SOD activity as a donor of electrons and reducing agent in various biological reactions in the body preventing the oxidation of lipids, proteins and DNA (González-Fuentes *et al.*, 2015; Estevinho *et al.*, 2016). There are reports of the use of AA for the treatment of *H. pylori* eradication in case of gastritis, may increase the effectiveness rate of treatment with PPIs and modulates oxidative effects of OME (Zojaji *et al.*, 2009).

However, the observed effects of RP and/or AA were more prominent with the modulation of up to 70% of OME-induced mutagenicity in *A. cepa*. There are reports that the association of vitamins has importance in the protection and inhibition of oxidative effects (Sun *et al.*, 2017) and prevention of risks for several types of cancer, such as adenomas (Xu *et al.*, 2013), colon (Heine-Bröring *et al.*, 2015), ovary (Koushik *et al.*, 2015), prostate (Tomasetti *et al.*, 2012) and especially for gastric cancer (Kong *et al.*, 2014).

5. Conclusions

OME in *A. cepa* induced genotoxic (DNA damage) and mutagenic (micronuclei, fragments, delays and chromosome losses) effects at all tested concentrations, while toxic by two largest concentrations and cytotoxicity with the highest concentration. Co-treatment with RP and/or AA inhibited and/or modulated the cytogenetics damages caused by OME, indicating these vitamins may have antigenotoxic, antimutagenic, antitoxic and anti-cytotoxic activities. These results suggest the prospects for preclinical and clinical cytogenetic studies with OME associated with vitamins.

To study the mutagenic effects of chemical compounds is an extremely important in the evaluation for the safety assessment, especially for the pharmaceutical products prior to the commencement of clinical trials.

6. References

Adeyemo, O.A., Farinmade, A.E., 2013. Genotoxicandcytotoxiceffects of food flavor enhancer, monosodium glutamate (MSG) using *Allium cepa* assay. Afric J. Biotechnol. 12, 1459-1466. doi: 10.5897/AJB12.2927

Al-Wajeeh, N.S., Hajrezaie, M., Al-Henhena, N., Kamran, S., Bagheri, E., Zahedifard, M., Saremi, K., Noor, S.M., Ali, H.M., Abdulla, M.A., 2017. The antiulcer effect of Cibotium barometz leaves in rats with experimentally induced acute gastric ulcer. Drug Des. Devel. Ther. 11, 995-1009. doi: 10.2147/DDDT.S107018.

Arai, A.E.; Gallerani, S.M.C., 2011. Uso Crônico de Fármacos Inibidores da Bomba de Prótons: Eficácia Clínica e Efeitos Adversos. Ph.D. Dissertation, Centro Universitário Filadéldia, Londrina, Brazil, 2011

Asaikkutti, A., Bhavan, P., Vimala, K., Karthik, M., Cheruparambath, P., 2016. Effect of different levels dietary vitamin C on growth performance, muscle composition, antioxidant and enzyme activity of freshwater prawn, Macrobrachium malcolmsonii. Aquacult. Rep. 3, 229-236. doi: 10.1016/j.aqrep.2016.04.002.

Asatryan, A.D., Komarova, N.L., 2016. Evolution of genetic instability in heterogeneous tumors. J. Theor. Biol. 396, 1-12. doi: 10.1016/j.jtbi.2015.11.028

Banala, R., Karnati, P., 2015. Vitamin A deficiency: An oxidative stress marker in sodium fluoride (NaF) induced oxidative damage in developing rat brain. Int. J. Dev. Neurosci. 47, 298-303. doi: 10.1016/j.ijdevneu.2015.08.010.

342

343 Brambilla, G., Maltiolli, F., Martelli, A., 2009. Genotoxic and carcinogenic effects of
344 antipsychotics and antidepressants. Toxicology 3, 77-88. doi: 10.1016/j.tox.2009.04.056

345

346 Burrell, R.A., Swanton, C., 2014. Tumour heterogeneity and the evolution of polyclonal drug
347 resistance. Mol. Oncol. 8, 1095-1111.

348

349 Cha, J., Yu, Q., Seo, J., 2016. Vitamin A supplementation modifies the antioxidant system in
350 rats. Nutr. Res. Pract. 10, 26. doi: 10.4162/nrp.2016.10.1.26.

351

352 Deshmukh, A.L., Kumar, C., Singh, D.K., Maurya, P., Banerjee, D., 2016. Dynamics of
353 replication proteins during lagging strand synthesis: A crossroads for genomic instability and
354 cancer. DNA Repair 42, 72-81. doi: 10.1016/j.dnarep.2016.04.010.

355

356 Downes, N., Foster, J., 2015. Regulatory Forum Opinion Piece: Carcinogen Risk
357 Assessment: The Move from Screens to Science. Toxicol. Pathol. 43, 1064-1073. doi:
358 10.1177/0192623315598578.

359

360 Estevinho, B., Carlan, I., Blaga, A., Rocha, F., 2016. Soluble vitamins (vitamin B12 and
361 vitamin C) microencapsulated with different biopolymers by a spray drying process. Powder
362 Technol. 289, 71-78. doi: 10.1016/j.powtec.2015.11.019.

363

364 Felisbino, S., Moreno, V., Batista, A.A., Silveira-Lacerda, E., 2014. Cytoxicity and apoptotic
365 mechanism of ruthenium (II) amino acid complexes in sarcoma-180 tumor cells. PLoS One,
366 9, 105-125.

Ferguson, L.R., Chen, H., Collins, A.R., Connell, M., Damia, G., Dasgupta, S., Malhotra, M., Meeker, A.K., Amedei, A., Amin, A., Ashraf, S.S., Aquilano, K., Azmi, A.S., Bhakta, D., Bilsland, A., Boosani, C.S., Chen, S., Ciriolo, M.R., Fujii, H., Guha, G., Halicka, D., Helferich, W.G., Keith, W.N., Mohammed, S.I., NIccolai, E., Yang, X., Honoki, K., Parslow, V.R., Prakash, S., Rezazadeh, S., Shackelford, R.E., Sidransky, D., Tran, P.T., Yang, E.S., MaxwelL, C.A., 2015. Genomic instability in human cancer: Molecular insights and opportunities for therapeutic attack and prevention through diet and nutrition. Semin. Cancer Biol. 35, S5-S24. doi: 10.1016/j.semcancer.2015.03.005

Heine-Bröring, R., Winkels, R., Renkema, J., Kragt, L., van Orten-Luiten, A., Tigchelaar, E., Chan, D., Norat, T., Kampman, E., 2015. Dietary supplement use and colorectal cancer risk: A systematic review and meta-analyses of prospective cohort studies. Int. J. Cancer 136, 2388-2401. doi: 10.1002/ijc.29277.

Herbet, M., Izdebska, M., Piątkowska-Chmiel, I., Poleszak, E., Jagiełło-Wójtowicz, E., 2016. Estimation of oxidative stress parameters in rats after simultaneous administration of rosuvastatin with antidepressants. Pharmacol. Rep. 68, 172-176. doi: 10.1016/j.pharep.2015.08.004.

Herrero, O., Pérez Martín, J.M., Fernández FreirE, P., Carvajal López, L., Peropadre, A., Hazen, M.J., 2012. Toxicological evaluation of three contaminants of emerging concern by use of the *Allium cepa* test. Mutat. Res. 743, 20-24. doi: 10.1016/j.mrgentox.2011.12.028

391 Islam, M.T., Streck, L., Alencar, M.V.O.B., Silva, S.W.C., Machado, K.C., Machado, K.C.,
392 Júnior, A.L.G., Paz, M.F.C.J., Mata, A.M.O.F., Sousa, J.M.C., Junior, J.S.C., Rolim, H.M.L.,
393 Silva-Junior, A.A., Melo-Cavalcante, A.A.C., 2017. Evaluation of toxic, cytotoxic and
394 genotoxic effects of phytol and its nanoemulsion. Chemosphere 177, 93-101. doi:
395 10.1016/j.chemosphere.2017.02.145.
396
397 Kohler, J.E., Blass, A.L., Liu, J., Tai, K., Soybel. D.I., 2010. Antioxidant pre-treatment
398 prevents omeprazole-induced toxicity in an in vitro model of infectious gastritis. Free Radic.
399 Biol. Med. 49, 786-791. doi: 10.1016/j.freeradbiomed.2010.05.034
400
401 Kong, P., Cai, Q., Geng, Q., Wang, J., Lan, Y., Zhan, Y., Xu, D., 2014. Vitamin Intake
402 Reduce the Risk of Gastric Cancer: Meta-Analysis and Systematic Review of Randomized
403 and Observational Studies. PLoS One 9, e116060. doi: 10.1371/journal.pone.0116060.
404
405 Kosma, C., Lambropoulou, D., Albanis, T., 2016. Analysis, occurrence, fate and risks of
406 proton pump inhibitors, their metabolites and transformation products in aquatic
407 environment: A review. Sci. Total Environ. 569-570, 732-750. doi:
408 10.1016/j.scitotenv.2016.06.160.
409
410 Koushik, A., Wang, M., Anderson, K., van den Brandt, P., Clendenen, T., Eliassen, A.,
411 Freudenheim, J., Genkinger, J., Håkansson, N., Marshall, J., McCullough, M., Miller, A.,
412 Robien, K., Rohan, T., Schairer, C., Schouten, L., Tworoger, S., Wang, Y., Wolk, A.,
413 Zeleniuch-Jacquotte, A., Smith-Warner, S., 2015. Intake of vitamins A, C, and E and folate
414 and the risk of ovarian cancer in a pooled analysis of 10 cohort studies. Cancer Causes
415 Control. 26, 1315-1327. doi: 10.1007/s10552-015-0626-0.

Lee, G., Lee, J., Lee, S., 2015. Antioxidant and Anticoagulant Status Were Improved by Personalized Dietary Intervention Based on Biochemical and Clinical Parameters in Cancer Patients. Nutr. Cancer 67, 1083-1092. doi: 10.1080/01635581.2015.1073754.

Lee, J., Kishikawa, M., Kumazoe, M., Yamada, K., Tachibana, H., 2010. Vitamin A Enhances Antitumor Effect of a Green Tea Polyphenol on Melanoma by Upregulating the Polyphenol Sensing Molecule 67-kDa Laminin Receptor. PLoS One 5, e11051. doi: 10.1371/journal.pone.0011051.

Lee, P., Vousden, K.H., Cheung, E.C., 2014. TIGAR TIGAR Burning Bright. Cancer Metab. 2, 1-9. doi: 10.1186/2049-3002-2-1

Lima, A.P.V.D., Filho, M.D.A.N., 2014. Efeitos em longo prazo de inibidores da bomba de prótons. Braz. J. Surg. Clin. Res. 5, 45-49.

Liman, R., 2013. Genotoxic effects of Bismuth (III) oxide nanoparticles by Allium and Comet assay. Chemosphere 93, 269-273. doi: 10.1016/j.chemosphere.2013.04.076

MELBOURNE, Austrália Publicado em Global Family Doctor 2011. Inibidores da bomba de prótons-efeitos adversos incomuns. Disponível em: http://www.sbmfc.org.br/default.asp?siteAcao=mostraPagi na&paginaId=748. Acesso em: 10 set. 2013.

Mesi, D. A., Kopliku, D., 2013. Cytotoxic and genotoxic potency screening of two pesticides on *Allium cepa* L. Procedia Technol. 8, 19-26. doi: 10.1016/j.protcy.2013.11.005

Moura, A.G., Santana, G.M., Ferreira, P.M.P., Sousa, J.M.C., Peron, A.P., 2016. Cytotoxicity of cheese and Cheddar cheese food fl avorings on *Allium cepa* L root meristems. Braz. J. Biol. 76, 439-443. doi: 10.1590/1519-6984.20514

Müerköster, S., Rausch, A.V., Isberner, A., Minkenberg, J., Blaszczuk, E., Witt, M., Fölsch, U.R., Schmitz, F., Schäfer, H., Arlt, A., 2008. The apoptosis-inducing effect of gastrin on colorectal cancer cells relates to an increased IEX-1 expression mediating NF-κB inhibition. Oncogene 27, 1122-1134. doi:10.1038/sj.onc.1210728

Murphy-Marshman, H., Quensel, K., Shi-Wen, X., Barnfield, R., Kelly, J., Peidl, A., Stratton, R.J., Leask, A., 2017. Antioxidants and NOX1/NOX4 inhibition blocks TGFβ1-induced CCN2 and α-SMA expression in dermal and gingival fibroblasts. PLoS One 12, e0186740. doi: 10.1371/journal.pone.0186740.

Novotna, A., Srovnalova, A., Svecarova, M., Korhonova, M., Bartonkova, I., Dvorak, Z., 2014. Differential Effects of Omeprazole and Lansoprazole Enantiomers on Aryl Hydrocarbon Receptor in Human Hepatocytes and Cell Lines. PLoS One 9, e98711. doi: 10.1371/journal.pone.0098711.

Özkara, A., Akyıl, D., Eren, Y., Erdoğmuş, S.F., 2015. Potential cytotoxic effect of Anilofos by using *Allium cepa* assay. Cytotechnology 67, 783-791. doi: 10.1007/s10616-014-9716-1

de Oliveira, M.R., 2015. Vitamin A and Retinoids as Mitochondrial Toxicants. Oxid. Med. Cell. Longev. 2015, 1-13. doi: 10.1155/2015/140267.

Palmeira, C., Lameiras, C., AmarO, T., Lima, L., Koch, A., Lopes, C., Oliveira, P.A., Santos, L., 2011. CIS is a surrogate marker of genetic instability and field carcinogenesis in the urothelial mucosa. Urol. Oncol. 29, 205-211. doi: 10.1016/j.urolonc.2009.07.022.

Paroni Sterbini, F., Palladini, A., Masucci, L., Cannistraci, C., Pastorino, R., Ianiro, G., Bugli, F., Martini, C., Ricciardi, W., Gasbarrini, A., Sanguinetti, M., Cammarota, G., Posteraro, B., 2016. Effects of Proton Pump Inhibitors on the Gastric Mucosa-Associated Microbiota in Dyspeptic Patients. Appl. Environ. Microbiol. 82, 6633-6644. doi: 10.1128/aem.01437-16.

Patlolla, J.M., Zhang, Y., Li, Q., Steele, V.E., Rao, C.V., 2012. Anti-carcinogenic properties of omeprazole against human colon cancer cells and azoxymethane-induced colonic aberrant crypt foci formation in rats. Int. J. Oncol. 40, 170-175. doi: 10.3892/ijo.2011.1214.

Paz, M.F.C.J., Alencar, M.B.O.V., Gomes Jr, A.L.G., Machado, K.C., Islam, M.T., Ali, E.S., Shill, M.C., Ahmed, M.I., Uddin, S.J., Mata, A.M.F., Carvalho, R.M., Sobral, A.P., Silva, F.C.C., Souza, J.M.C., Cavalcante, A.M.C.M., Mishra, S.K., Silva, J., 2018. Correlations between risk factors for breast cancer and genetic instability in cancer patients - a clinical perspective study. Front. Genet. 8, 236. DOI: 10.3389/fgene.2017.00236.

Persaud, S.D., Park, S.W., Ishigami-Yuasa, M., Koyano-Nakagawa, N., Kagechika, H., Wei, L. N., 2016. All trans-retinoic acid analogs promote cancer cell apoptosis through non-

genomic Crabp1 mediating ERK1/2 phosphorylation. Scientific reports, 6, article number: 22396. doi:10.1038/srep22396

Portantiolo, T.N., Vale, I.A.V.D., Bergmann, R.B., Abib, R.T., 2014. Consumo de Vitaminas Antioxidantes por Mulheres com Câncer de Mama submetidas ao Tratamento Quimioterápico na Cidade de Pelotas-RS. Rev. Bras. Cancerol. 60, 4, 323-329.

Potapova T. and Gorbsky G.J., 2017. The Consequences of Chromosome Segregation Errors in Mitosis and Meiosis. Biology (Basel), 6(1): 12-33.

Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., 2014. Oxidative stress, prooxidants, and antioxidants: the interplay. Biomed. Res. Int., 1-19. doi: 10.1155/2014/761264

Rouphael, Y., Raimondi, G., Lucini, L., Carillo, P., Kyriacou, M.C., Colla, G., Cirillo, V., Pannico, A., El-Nakhel, C., De Pascale, S., 2018. Physiological and Metabolic Responses Triggered by Omeprazole Improve Tomato Plant Tolerance to NaCl Stress. Front Plant Sci. 9, 249. doi: 10.3389/fpls.2018.00249.

Seoane, M., Esperanza, M., Cid, Á., 2017. Cytotoxic effects of the proton pump inhibitor omeprazole on the non-target marine microalga Tetraselmis suecica. Aquat. Toxicol. 191, 62-72. doi: 10.1016/j.aquatox.2017.08.001.

Shao, L., Zhu, X., Yang, Y., Jin, J., Liu, H., Han, D., Xie, S., 2016. Effects of dietary vitamin A on growth, hematology, digestion and lipometabolism of on-growing gibel carp (Carassius

auratus gibelio var. CAS III). Aquaculture. 460, 83-89. doi: 10.1016/j.aquaculture.2016.03.054

Sharma, S., Vig, A.P., 2012. Genotoxicity of Atrazine, Avenoxan, Diuron and Quizal of op-P-ethyl Herbicides using the *Allium cepa* Root Chromosomal Aberration Assay. Terr. Aquat. Environ. Toxicol. 6, 90-95.

Sharma, S., Venkatesan, V., Prakhya, B. M., Bhonde, R., 2015. Human mesenchymal stem cells as a novel platform for simultaneous evaluation of cytotoxicity and genotoxicity of pharmaceuticals. Mutagenesis. 30, 391-399. doi: 10.1093/mutage/geu086.

Sun, W., Zhai, M., Zhou, Q., Qian, C., Jiang, C., 2017. Effects of B Vitamins Overload on Plasma Insulin Level and Hydrogen Peroxide Generation in Rats. Chin. J. Physiol. 60, 207-214. doi: 10.4077/CJP.2017.BAF469.

Thomson, A.B.R., Sauve, M.D., Kassam, N., Kamitakahara, H., 2010. Safety of the long-term use of proton pump inhibitors. World J. Gastroenterol. 16, 2323-2330. doi: 10.3748/wjg.v16.i19.2323.

Tomasetti, M., Nocchi, L., Neuzil, J., Goodwin, J., Nguyen, M., Dong, L., Manzella, N., Staffolani, S., Milanese, C., Garrone, B., Alleva, R., Borghi, B., Santarelli, L., Guerrieri, R., 2012. Alpha-tocopheryl succinate inhibits autophagic survival of prostate cancer cells induced by vitamin K3 and ascorbate to trigger cell death. PLoS One 7, e52263. doi: 10.1371/journal.pone.0052263.

Tu¨rkog˘lu S., 2012. Determination of genotoxic effects of chlorfenvinphos and enbuconazole in *Allium cepa* root cells by mitotic activity chromosome aberration DNA content and comet assay. Pest. Biochem. Physiol. 103, 224-230. doi: 10.1016/j.pestbp.2012.06.001

Wadhwa, R., Song, S., Lee, J., Yao, Y., Wei, Q., Ajani, J., 2013. Gastric câncer-molecular and clinical dimensions. Nat. ver. Clin. Oncol. 10, 643-655. doi: 10.1038/nrclinonc.2013.170.

Xu, X., Yu, E., Liu, L., Zhang, W., Wei, X., Gao, X., Song, N., Fu, C., 2013. Dietary intake of vitamins A, C, and E and the risk of colorectal adenoma. Eur. J. Cancer Prev. 22, 529-539. doi: 10.1097/cej.0b013e328364f1eb.

Yanagihara, G., de Paiva, A., Neto, M., Torres, L., Shimano, A., Louzada, M., Annoni, R., Penoni, Á., 2015. Efeitos da administração em longo prazo do omeprazol sobre a densidade mineral óssea e as propriedades mecânicas do osso. Rev. Bras. Ortop. 50, 232-238. doi: 10.1016/j.rbo.2014.05.012.

Yildiz, M.; Cig˘erci, I.H.; Konuk, M.; Fidan, A.F.; Terzi, H., 2009. Determination of genotoxic effects of copper sulphate and cobalt chloride in *Allium cepa* root cells by chromosome aberration and comet assays. Chemosphere 75, 934-938. doi: 10.1016/j.chemosphere.2009.01.023.

Zeiger, E., Gollapudi, B., Aardema, M.J., Auerbach, S., Boverhof, D., Custer, L., 2015. Opportunities to integrate new approaches in genetic toxicology: an ILSI-HESI workshop report. Environ. Mol. Mutagen. 56, 277-285. doi: 10.1002/em.21923.

Zhang, Q., Huang, N., Wang, J., Luo, H., He, H., Ding, M., Deng, W.Q., Zou, K., 2013. The H+/K+-ATPase inhibitory activities of Trametenolic acid B from Trametes lactinea (Berk.) Pat, and its effects on gastric cancer cells. Fitoterapia 89, 210-217. doi: 10.1016/j.fitote.2013.05.021.

Zojaji, H., Talaie, R., Mirsattari, D., Haghazali, M., Molaei, M., Mohsenian, N., Derakhshan, F., Zali, M., 2009. The efficacy of Helicobacter pylori eradication regimen with and without vitamin C supplementation. Dig. Liver Dis. 41, 644-647. doi: 10.1016/j.dld.2008.09.008.

Conflicts of interest

None declared.

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587 **Table 1.** Genotoxicity of OME and modulatory effects of RP and/or AA on the meristematic
588 cells of *A. cepa* (comet assay)
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Treatment groups	ID	FD
NC	48.0 ± 1.4	18.5 ± 0,7
PC	198.0 ± 3.7	91.0 ± 1.8
OME 10 µg/mL	100.0 ± 6.7[ab]	45.5 ± 0.7[ab]
OME 20 µg/mL	111.0 ± 2.0[ab]	50.5 ± 3.5[ab]
OME 40 µg/mL	149.5 ± 6.3[ab]	65.0 ± 1.4[ab]
OME 10 µg/mL + RP	81.4 ± 1.4[b]	42.0 ± 1.4[ab]
OME 20 µg/mL + RP	86.0 ± 2.6[abc]	50.0 ± 1.4[ab]
OME 40 µg/mL + RP	106.5 ± 5.6[abd]	59.5 ± 2.1[ab]
OME 10 µg/mL + AA	83.0 ± 4.2[bc]	42.5 ± 0.7[ab]
OME 20 µg/mL + AA	96.5 ± 10.6[ab]	50.5 ± 2.1[ab]
OME 40 µg/mL + AA	103.5 ± 6.3[ad]	54.5 ± 3.5[ab]
OME 10 µg/mL + RP + AA	73.0 ± 4.2[bc]	38.5 ± 0.7[ab]
OME 20 µg/mL + RP + AA	78.5 ± 4.9[bc]	40.2 ± 1.4[abc]
OME 40 µg/mL + RP + AA	84.0 ± 7.0[bd]	52.0 ± 1.4[abd]

Values are mean ± SD (n = 5), ANOVA one-way and Bonferroni post-test, significant values $p < 0.05$ when compared to the [a]NC; [b]OME 10 µg/mL; [c]OME 20 µg/mL and [d]OME 40 µg/mL; RP, retinol palmitate (55 µg/mL); AA, ascorbic acid (352.2 µg/mL); PC, positive control (copper sulphate at 0.6 µg/mL); NC, negative control (dechlorinated water).

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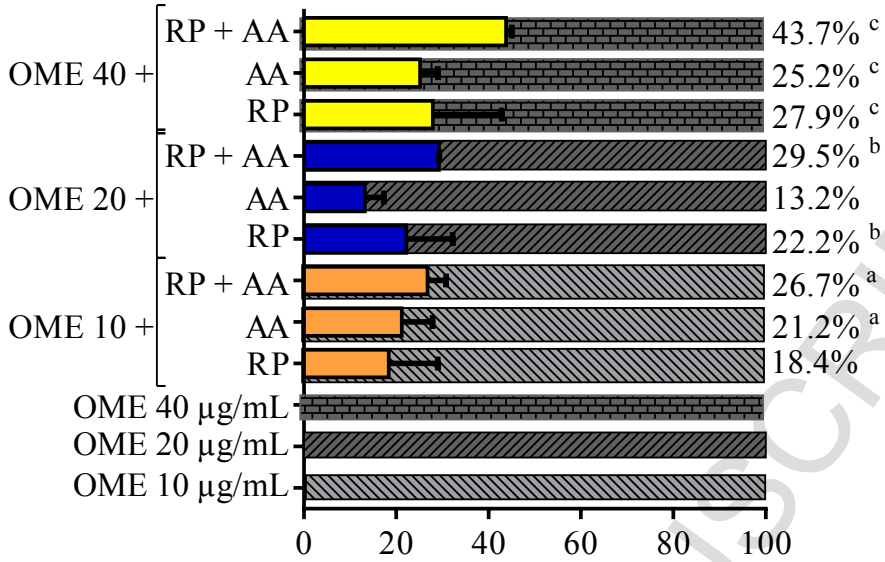


Figure 1. Effects of RP and/or AA on OME-induced genotoxic damage to the meristematic cells of *A. cepa* (comet assay) [Values are percentage modulation (n = 5), ANOVA one-way Tukey post-test, significant values: $p < 0.05$ when compared tothe [a]OME 10 µg/mL, [b]OME 20 µg/mL and [c]OME 40 µg/mL].

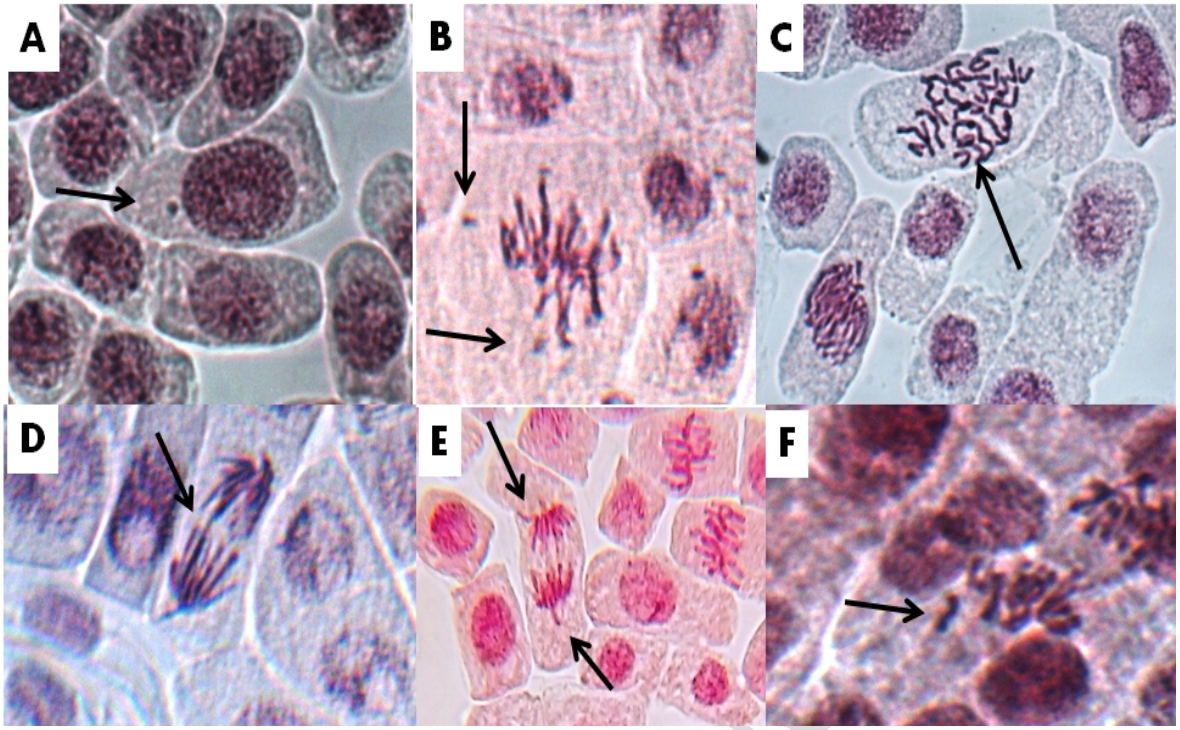
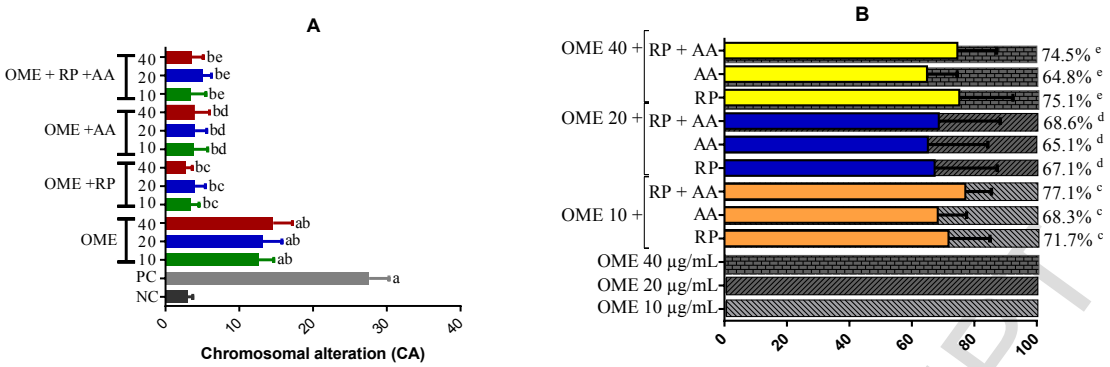


Figure 2. Photomicrography of mutagenic effects of OME (40 µg/mL) on *A. cepa* meristematic cells. [Coloration with acetic Carmine, magnification at 400X under the optical microscope. A: MN in prophase; B: chromosomal breaks in metaphase; C: c-metaphase; D: chromosome bridge in anaphase; E: chromosomal delays; F: chromosome loss in anaphase].



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619 **Figure 3.** Mutagenicity of OME and the modulatory effects of RP and/or AA in *A. cepa.*
620 [Values are mean ± SD and percentage, (n = 5), ANOVA one-way and Bonferroni post-test,
621 significant values of p <0.05 when compared to the [a]NC, [b]PC, [c]OME 10 µg/mL, [d]OME 20
622 µg/mL and [e]OME 40 µg/mL; RP, retinol palmitate (55 µg/mL); AA, ascorbic acid (352.2
623 µg/mL); PC, positive control (copper sulphate at 0.6 µg/mL); NC, negative control
624 (dechlorinated water).]

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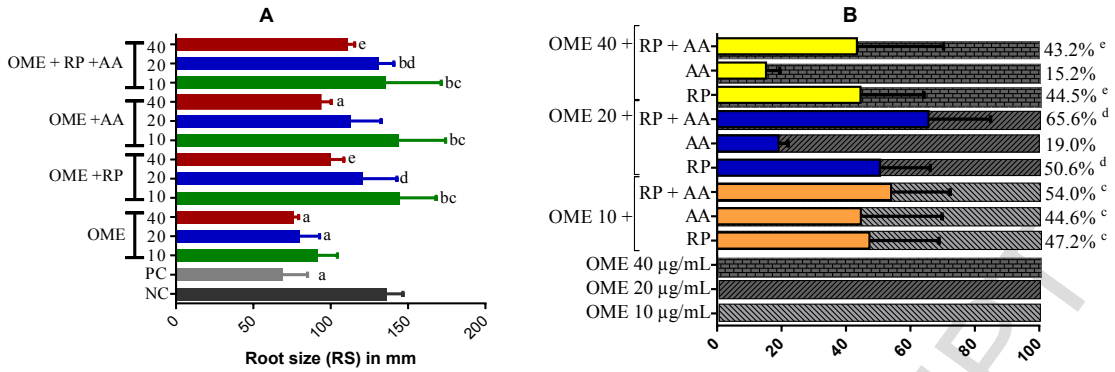

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635 **Figure 4.** Toxicity of OME and the effects of RP and/or AA in *A. cepa* [Values are mean ±
636 SD and percentage, (n = 5), ANOVA one-way and Bonferroni post-test, significant values of
637 $p < 0.05$ when compared to the [a]NC, [b]PC, [c]OME 10 µg/mL, [d]OME 20 µg/mL and [e]OME 40
638 µg/mL; RP, retinol palmitate (55 µg/mL); AA, ascorbic acid (352.2 µg/mL); PC, positive
639 control (copper sulphate at 0.6 µg/mL); NC, negative control (dechlorinated water).]

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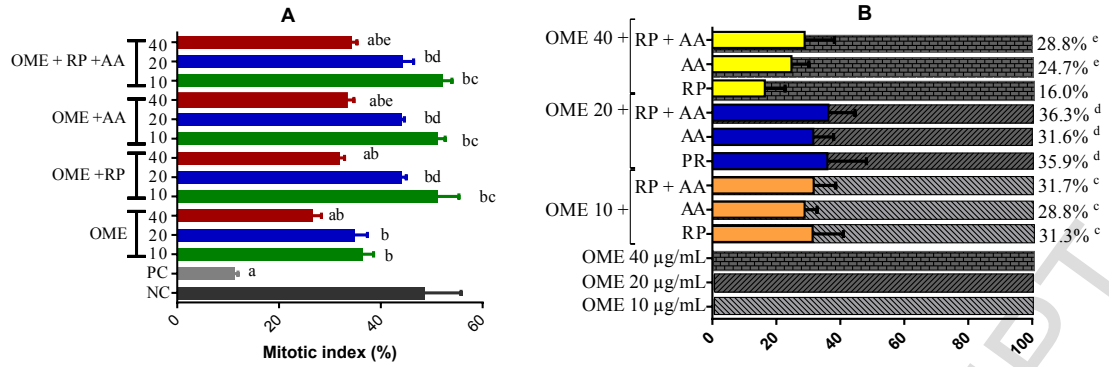

Figure 5. Cytotoxicity of OME and the effects of RP and/or AA in *A. cepa* root meristems [Values are mean ± SD and percentage, (n = 5), ANOVA one-way and Bonferroni post-test, significant values of $p < 0.05$ when compared to the [a]NC, [b]CP, [c]OME 10 µg/mL, [d]OME 20 µg/mL and [e]OME 40 µg/mL; RP, retinol palmitate (55 µg/mL); AA, ascorbic acid (352.2 µg/mL); PC, positive control (copper sulphate at 0.6 µg/mL); NC, negative control (dechlorinated water).]

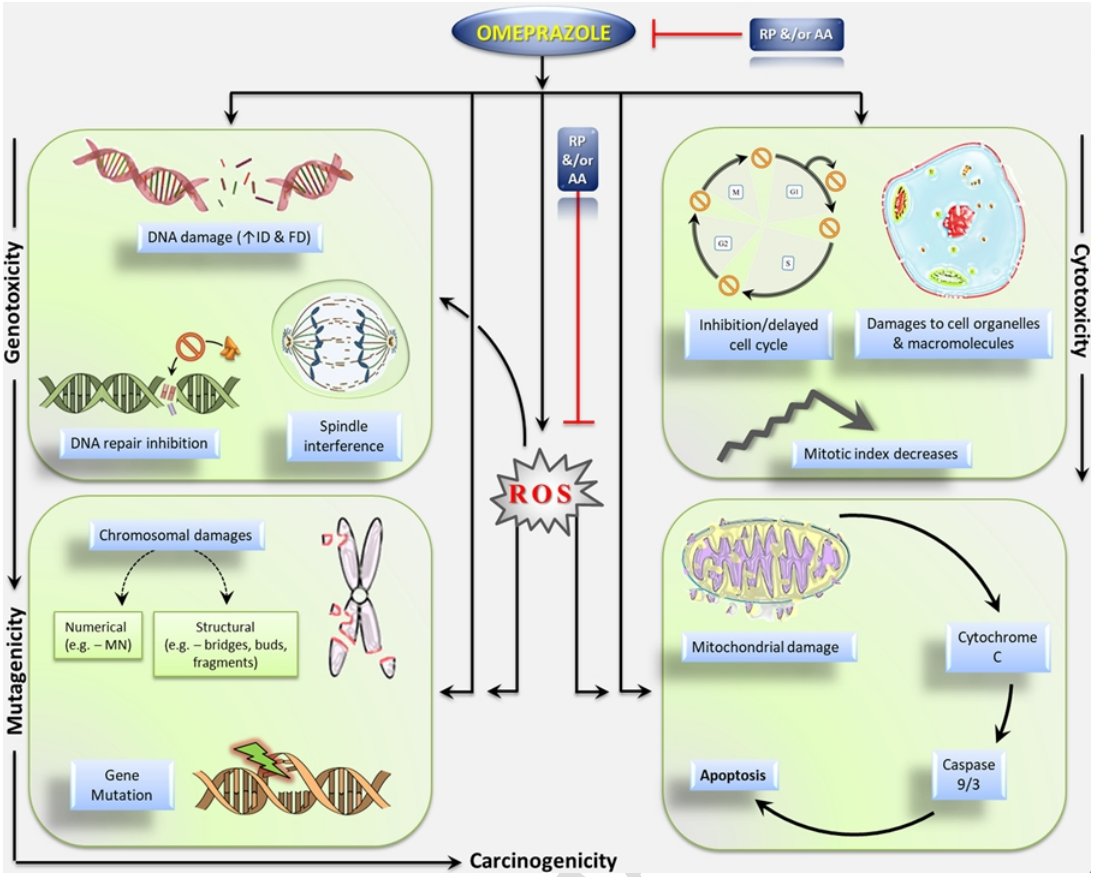

Figure 6. Possible cytogenetic events of OME in eukaryotic system based on *A. cepa* test. [Omeprazole (OME) by inhibiting or delaying the cell cycle, damaging cell organelles and cellular macromolecules such as carbohydrates, proteins, lipids may induce cytoxicity. Moreover, by inducing reactive oxygen species (ROS), it may damage mitochondria, leading to release cytochrome *C* and activates caspase 9/3, resulting apoptotic cell death. OME and OME-induced oxidative stress can cause damage of the genetic materials (e.g. DNA and RNA), inhibit the repair capacity, and cause spindle interference, result in genotoxic effects. Furthermore, mutagenicity may occur by OME-induced chromosomal damage and gene mutation in the host cells. Finally, the genotoxicity may turn to mutagenicity, eventually to the carcinogenicity. Retinol palmitate (RP) and/or ascorbic acid (AA) may inhibit or interfere overall cytogenetic processes caused by OME especially as antioxidants they can act against ROS. However, both antioxidants can also modulate the cytogenetic damages caused by OME. ID, index of damage; FD, frequency of damage; MN, micronuclei.]

Highlights

- Omeprazole induced toxic, cytotoxic, genotoxic and mutagenic effects in *Allium cepa*.
- Retinol palmitate and ascorbic acid inhibited/modulted omeprazole's activity.
- Antioxidant vitamins during omeprazole-mediated toxicity.