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CELLULAR AND MOLECULAR BIOLOGY

Protective effect of kavain in meristematic cells of *Allium cepa* L.

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Abstract: Kavain is one of the main kavalactones of *Piper methysticum* (Piperaceae) with anxiolytic, analgesic, and antioxidant activities. Therefore, the aim of the study was to evaluate the cytotoxic, mutagenic, and antimutagenic potential of kavain in *Allium cepa* cells. Roots of *A. cepa* were transferred to the negative (2% acetone) and positive (10 µg/mL of Methylmethanesulfonate, MMS) controls and to the concentrations of kavain (32, 64 and 128 µg/mL) for 48 h. A total of 5,000 meristematic cells were analyzed under an optical microscope to determine the mitotic index, mean number of chromosomal alterations and percentage of damage reduction. Data were analyzed by Kruskal-Wallis test (p <0.05). All concentrations of kavain were not cytotoxic and did not show significant chromosomal changes when compared to 2% acetone. Kavain showed a cytoprotective effect in the pre (128 µg/mL) and in the post-treatment (32 and 64 µg/mL) and reduced damage against the mutagenic action of MMS in all concentrations of the pre and simultaneous and at the highest of post (128 µg/mL). Kavain promoted a significant reduction in micronuclei, nuclear buds and chromosomal losses in relation to MMS. The observed data indicate the importance of kavain for the inhibition of damage and chemoprevention.

Key words: *Allium cepa*, chemoprotection, chromosome alterations, Kavalactona.

INTRODUCTION

Medicinal plants have antifungal, antimicrobial, insecticidal, and antiseptic activities (Hosseinzadeh et al. 2015), and are used in many different countries and cultures for the treatment of urinary tract infections, epilepsy and diabetes, playing a key role in research on herbal medicines and the development of new drugs (Asadbeigi et al. 2014, Dias et al. 2014, Aragão et al. 2015). In addition, studies show that approximately 80% of the world population uses medicines of plant origin (Delfan et al. 2014), however they are still used empirically by the population and can cause toxic effects (Bae et al. 2015).

Piper methysticum G. Forster is a perennial shrub of the family Piperaceae known as Kava, kava-kava or awa (Einbonda et al. 2017). Originating from the Pacific Ocean islands, the infusion prepared from dry roots and rhizomes is traditionally used by island communities in religious rituals to induce a relaxed psychological state (Singh & Singh 2002, Lebot & Legendre 2016). In Europe, it is marketed without prescription, as an alternative to benzodiazepines to treat anxiety and insomnia (Chua et al. 2016).

Kava has in its chemical composition several constituents; the main ones are called kavapyrones or kavalactones (Ketola et al. 2015) present mainly in the rhizome (Singh & Singh 2002). In total, 18 kavalactones have

been identified, with an emphasis on kavain, yangonin, desmethoxyyangonin, dihydrokavain, methysticin and dihydromethysticin that have greater pharmacological importance (Kuchta et al. 2017); and kavain is present in greater amount in kava extracts (Chua et al. 2016).

Kavain is part of a group of α-pyrone, isolated for the first time as an isomer from the root and rhizome of *P. methysticum*. It has a trans-double bond that connects the phenyl and lactone rings and a single C6 stereogenic center (Cirilli et al. 2008). Studies have shown that kavain has analgesic and anxiolytic activity (Wang et al. 2018, Chua et al. 2016), as well as anti-epileptic (Grunze et al. 2001), antioxidant (Singh et al. 2018), anti-inflammatory (Tang & Amar 2016), antithrombotic (Gleitz et al. 1997), anticonvulsant (Gleitz et al. 1996), neuroprotective (Wruck et al. 2008) activities, and potential to treat osteolytic diseases (Guo et al. 2018). Kavain also has antitumor activity by inhibiting the nuclear factor-κB (NF-κB) of human pulmonary adenocarcinoma cells (IC_{50} = 32 ± 3 µg/mL) and low toxicity (IC_{50} = 166 ± 14 µg/mL) to Hepa1c1c7 liver cells (Shaik et al. 2009). Silva et al. (2021) showed that kavain had no mutagenic and/or recombinogenic effect (32, 64 and 128 µg/mL) and was antimutagenic at the lowest concentration (32 µg/mL) in tests performed with *Drosophila melanogaster*.

Kavain and other kavalactones alone or combined with the extract of kava have the capacity to inhibit several isoforms of cytochrome P450 (CYP450), being: CYP1A2, CYP2C9; CYP2C19; CYP2D6, CYP3A4; CYP4A9 and CYP4A9/11 (Mathews et al. 2005). This property is the source of numerous interactions, mainly pharmacokinetic, with other drugs, as it decreases their metabolism by inhibiting enzymes of the CYP450 complex, which can induce toxicity (Zou et al. 2004, Mathews et al. 2002). However, evidence of pharmacokinetic and/or pharmacodynamic interactions remains unsustainable and only a few investigations have been carried out on the potential of kava and kavalactone preparations to interact with specific drugs.

Studies on the medicinal potential of kavain and its interaction with DNA and spindle fibers are important for safety in its administration. The *Allium cepa* L. (onion) test system has often been used to monitor the toxicogenetic activity of isolated compounds (Luz et al. 2012, Liman et al. 2019, Shetty et al. 2017, De Souza et al. 2017). The test system for chromosomal changes in *A. cepa* is widely cited in the literature as a bioindicator for the evaluation of cytotoxicity, genotoxicity and protective effect of chemical compounds, as it has rapid cell multiplication, large and few chromosomes, which allows better analysis of structural and numerical alterations (Bonciu et al. 2018, Leme & Marin-Morales 2009). The *A. cepa* bioassay stands out for being an excellent bioindicator of mutagenic compounds, has low cost, reliability and agreement with other test organisms, helping studies to prevent damage to human health (Oliveira et al. 2013, Firbas & Amon 2014, Kumar et al. 2015, Liman et al. 2015). According to Rank & Nielsen (1997) and Fedel-Miyasato et al. (2014), a comparison of the mutagenicity assays in *A. cepa* with tests in rodents shows a agreement of approximately 80%, and therefore allows the confirmation of the mutagenic and antimutagenic mode of action. Besides that, it has a good correlation with cytotoxicity and genotoxicity tests *in vitro* or *in vivo* (Eren & Özata 2014, Sá et al. 2019).

A. cepa is also used to assess the reduction of DNA damage (Mauro et al. 2014). A chemoprotection can be desmutagenic or bio-antimutagenic. Desmutagenic substances neutralize mutagenic agents either directly or through their derivatives to inactivate them chemically in the extra- or intracellular medium

(Fedel-Miyasato et al. 2014, Felicidade et al. 2014). Bio-antimutagenic agents stimulate repair and replication of DNA and act at the cellular level by increasing reliability of replication, error-free repair and inhibiting repair systems that are subject to error (Oliveira et al. 2006).

With this in mind, the present study aimed to evaluate the mutagenic and antimutagenic potential of this molecule in meristematic cells of *A. cepa*.

MATERIALS AND METHODS

Chemical agent

The tested substance was DL-kavain, CAS 3155-48-4, molecular formula $C_{14}H_{11}O_3$ and molecular weight of 230.26 g/mol produced by Sigma-Aldrich Brasil Ltda. The preparations of Kavain (32, 64 and 128 µg/mL) and Methylmethanesulfonate (MMS, CAS 66-27-3, Sigma-Aldrich Brasil Ltda) were diluted in a solution of 2% acetone (Acetona PA; Dinâmica Química Contemporânea Ltda) and ultrapure water, obtained from the MilliQ system (Millipore, Vimodrone, Milan, Italy).

DNA-damaging agent

The MMS was used to induce DNA damage in meristematic cells of *A. cepa*. MMS (10 µg/ mL) is an alkylating agent with direct activity, inducing disturbances such as DNA breaks, bridges and chromosome loss, which are also expressed as micronuclei (Bianchi et al. 2016, Couto et al. 2019).

A. cepa test

One hundred seeds of *A. cepa* were germinated at the Genetics Laboratory of FACIME at room temperature in Petri dishes containing filter paper moistened with distilled water. The seeds with roots approximately 2 cm long were subjected to different treatments to evaluate mutagenicity and antimutagenicity according to Couto et al. (2019) and Pereira et al. (2020).

To assess mutagenicity, 30 seeds were transferred to the negative control (NC = acetone 2%), solvent (distilled water), positive control I (PC I = 10 µg/mL of MMS solubilized in distilled water), positive control II (PC II = 10 µg/mL of MMS solubilized in acetone 2%) and 32, 64 and 128 µg/mL concentrations of Kavain in separate dishes for each control and concentration. The Kavain concentrations used in this study were pre-determined based on the non-mutagenic effect in *D. melanogaster* (Silva et al. 2021).

Three protocols were established to evaluate antimutagenicity using the DNA damaging agent, MMS: pretreatment to assess preferentially desmutagenic action, simultaneous treatment to assess desmutagenic and bio-antimutagenic activity, and post-treatment to assess bio-antimutagenic action (Fedel-Miyasato et al. 2014, Couto et al. 2019, Mauro et al. 2014, Rocha et al. 2016, Pereira et al. 2020).

For the pretreatment group, the seeds were transferred to 32, 64 and 128 µg/mL concentrations of Kavain for 24 h, then to MMS solution for additional 24 h. For the simultaneous treatment, the seeds were transferred to ultrapure water for 24 h, then transferred to the 32, 64 and 128 µg/mL concentrations of Kavain and MMS solution at the same time for an additional 24 h. For the post-treatment group, the seeds were grown in MMS for 24 h and germinated for an additional 24 h in 32, 64 and 128 µg/mL of Kavain.

After mutagenic and antimutagenic treatments, the root tips were fixed in 3:1 methanol:acetic acid and stored at -20 ºC until slide preparation. The root tips were washed in distilled water three times for 5 min each, then hydrolyzed at 60 ºC for 10 min in 1 N HCl. After hydrolysis, the root tips were washed again in distilled water, transferred to amber glass bottles containing Schiff's reagent and kept there for 2

h in the dark. The root tips then were washed until the reagent was removed, transferred onto slides, squashed with one drop of 2% acetic carmine and mounted with Entellan® (107960; Merck Millipore) (Almeida et al. 2015).

The mitotic index (MI) indicates cytotoxicity and chromosome alterations, which reflects mutagenicity. To determine the MI, the number of cells in different phases of mitosis was divided by the total number of cells. For chromosome alterations, the number of alterations was divided by the total number of cells. We scored 5,000 meristematic cells on ten slides/treatment using light microscopy at 400 x magnification (Olympus CX 21, Zhejiang, China). Chromosome alterations included those resulting from aneugenic activity, e.g., C-metaphases, metaphases with chromosome adherence, lost chromosomes, multipolar anaphases, binucleate cells and polyploid metaphases, or clastogenic effects, e.g., chromosome fragments in metaphase or anaphase and chromosome bridges. MN may arise from either aneugenic or clastogenic effects (Anacleto et al. 2017).

Antimutagenic activity was assessed the percentage of damage reduction (%DR). The %DR was calculated for each treatment using the formula: %DR = [(a - b)/(a - c)] x 100 where a = number of damaged cells in the PC, b = number of damaged cells in each treatment, c = number of damaged cells in the NC (Waters et al. 1990).

Statistical analysis

Data were evaluated using the nonparametric test of Kruskal-Wallis followed by the *post hoc* test of Student-Newman-Keuls using the program, BioEstat 5.3 (Ayres et al. 2007). Values for $p \leq 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Recent studies have focused on the identification of phytochemicals/isolated compounds with beneficial effects, and on the elucidation of mechanisms that are related to protective action in the cell (Qian et al. 2016, Sharma et al. 2012, Zhang et al. 2016). This reinforces the concern of researchers in the search and development of new drugs that are more efficient against cancer, more effective in protecting and repairing DNA and preventing the formation of tumors (Słoczyńska et al. 2014). Therefore, considering the medicinal importance of kavain and the need for more toxicogenetic information, the present study aimed to investigate the cytotoxic, mutagenic and antimutagenic effect of this molecule on the meristematic cells of *A. cepa*.

The results of the present study showed that all kavain concentrations (32, 64 and 128 µg/mL) were not cytotoxic, as there was no significant reduction in the mitotic index (MI) of *A. cepa* cells in relation to the negative control (2% acetone) (Table I). Thus, kavain allowed the progression of the cell cycle of *A. cepa*, which reinforces its non-interference in DNA synthesis and/or in the inhibition of the G1/S and G2/M checkpoints in cells of *A. cepa*, as proposed by Mauro et al. (2014) with the inulin isolate. The non-cytotoxic effect has also been found in previous studies on kavain in human liver hepatocellular carcinoma cells (HepG2) using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], lactate dehydrogenase (LDH) and ethidium bromide (EB) (Tang et al. 2011) assays in hippocampus cells (Mulholland & Prendergast 2002), in astrocytoma and bone cells of rats, using the LDH and MTS assays (Wruck et al. 2008, Guo et al. 2018). In these studies, the concentrations were lower (ranging from 0.23 to 23 µg/mL), however even at higher concentrations (IC_{50} = 166 ± 14 µg/mL), kavain also showed low toxicity to Hepa1c1c7 liver cells (Shaik et al. 2009).

Table I. Mitotic index, total chromosomal alterations and percentage of damage reduction (%DR) in meristematic cells of *A. cepa*.

Treatment	Mitotic index	Chromosomal alteration	%DR
Mutagenicity			
NC	38.79 ± 13.67	1.62 ± 0.95	
MMS	18.20 ± 9.67**	24.15 ± 11.74**	
Kavain			
32 µg/mL	39.66 ± 18.33	0.18 ± 0.17	
64 µg/mL	32.18 ± 18.77	0.82 ± 0.40	
128 µg/mL	27.86 ± 10.55	0.81 ± 0.42	
Antimutagenicity			
Pretreatment (Kavain +MMS)			
32 µg/mL + 10 µg/mL	27.47 ± 10.01	5.79 ± 3.96^{+}	81.49
64 µg/mL + 10 µg/mL	27.17 ± 12.05	2.80 ± 1.38^{++}	94.76
128 µg/mL + 10 µg/mL	37.01 ± 8.18^{++}	3.83 ± 1.32^{+}	90.19
Simultaneous treatment			
(Kavain +MMS)			
32 µg/mL + 10 µg/mL	25.32 ± 9.28	4.88 ± 2.88^{+}	85.53
64 µg/mL + 10 µg/mL	16.79 ± 5.40	1.96 ± 1.26^{++}	98.49
128 µg/mL + 10 µg/mL	23.47 ± 10.28	1.50 ± 1.58^{++}	100.53
Post-treatment (MMS + Kavain)			
10 µg/mL +32 µg/mL	38.57 ± 11.57^{++}	14.77 ± 8.25	41.63
10 µg/mL + 64 µg/mL	31.67 ± 17.41^{+}	11.33 ± 5.38	56.90
10 µg/mL + 128 µg/mL	24.72 ± 12.06	4.25 ± 2.43^{++}	88.33

Data are means ± SD. NC, negative control (acetone 2%); MMS, methyl methanesulfonate (10 µg/mL, positive control); pre-treatment, 24 h Kavain + 24 h MMS; simultaneous treatment, 24 h distilled water + 24 h combined Kavain and MMS; post-treatment, 24 h MMS + 24 h Kavain. *Significant difference for the Kavain compared to NC. $^{+}$Significant difference for pre, simultaneous and post-treatment compared to MMS. Significant by Kruskal-Wallis test with a posteriori Student-Newman-Keuls test (* $p < 0.05$; $^{**/++}$ $p < 0.01$). Data are for 5,000 cells/treatment. The acetone 2% was used as a negative control, but how the results were statistical identical to solvent (distilled water), the data using water were omitted. The positive control I (PC I = 10 µg/mL of MMS solubilized in distilled water) and positive control II (PC II = 10 µg/mL of MMS solubilized in acetone 2%) also were statistical identical, the data using PC I were omitted.

All kavain concentrations were not mutagenic (total mean chromosomal changes) to *A. cepa* cells, as the mean values were not significantly different from the negative control (NC) (Table I). In addition, all chromosomal changes assessed individually were also not significant, except for micronuclei (MN) with lower mean values than NC (Table II) (Figure 1), showing that kavain did not interfere with the chromatin condensation processes, polymerization of mitotic spindle

Table II. Chromosomal alterations in meristematic cells of *A. cepa*.

Treatment	Chromosomal alteration					
Mutagenicity	**Cm**	**CL**	**NB**	**MN**	**CB**	**CF**
NC	0.0 ± 0.0	0.0 ± 0.0	0.34 ± 0.24	1.10 ± 1.01	0.18 ± 0.26	0.0 ± 0.0
MMS	0.25 ± 0.15	2.08 ± 0.41**	1.41 ± 0.08**	19.49 ± 11.34**	0.26 ± 0.16	0.0 ± 0.0
Kavain						
32 µg/mL	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0**	0.09 ± 0.19	0.08 ± 0.17
64 µg/mL	0.0 ± 0.0	0.0 ± 0.0	0.09 ± 0.18	0.46 ± 0.44**	0.27 ± 0.22	0.0 ± 0.0
128 µg/mL	0.0 ± 0.0	0.0 ± 0.0	0.26 ± 0.11	0.55 ± 0.40**	0.0 ± 0.0	0.0 ± 0.0
Antimutagenicity						
Pretreatment (Kavain +MMS)						
32 µg/mL + 10 µg/mL	0.10 ± 0.30	0.0 ± 0.0^{++}	0.16 ± 0.15^{++}	5.15 ± 2.59^{+}	0.09 ± 0.29	0.29 ± 0.24
64 µg/mL + 10 µg/mL	0.0 ± 0.0	0.0 ± 0.0^{++}	0.55 ± 0.67	1.88 ± 1.50^{++}	0.29 ± 0.25	0.08 ± 0.16
128 µg/mL + 10 µg/mL	0.0 ± 0.0	0.10 ± 0.31^{++}	0.28 ± 0.16^{+}	3.44 ± 1.07^{+}	0.0 ± 0.0	0.0 ± 0.0
Simultaneous treatment						
(Kavain +MMS)						
32 µg/mL + 10 µg/mL	0.08 ± 0.26	0.0 ± 0.0^{++}	0.29 ± 0.26^{+}	4.26 ± 2.58^{+}	0.17 ± 0.15	0.09 ± 0.19
64 µg/mL + 10 µg/mL	0.08 ± 0.24	0.0 ± 0.0^{++}	0.27 ± 0.20^{+}	1.61 ± 1.31^{++}	0.0 ± 0.0	0.0 ± 0.0
128 µg/mL + 10 µg/mL	0.0 ± 0.0	0.0 ± 0.0^{++}	0.38 ± 0.25^{+}	0.93 ± 0.55^{++}	0.10 ± 0.11	0.10 ± 0.11
Post-treatment (MMS + Kavain)						
10 µg/mL +32 µg/mL	0.0 ± 0.0	0.19 ± 0.29^{++}	0.38 ± 0.28+	13.84 ± 8.16	0.19 ± 0.29	0.18 ± 0.28
10 µg/mL + 64 µg/mL	0.0 ± 0.0	0.10 ± 0.11^{++}	0.09 ± 0.18^{++}	9.91 ± 2.25	0.38 ± 0.39	0.86 ± 0.33
10 µg/mL + 128 µg/mL	0.09 ± 0.19	0.0 ± 0.0^{++}	0.20 ± 0.12^{++}	3.87 ± 2.51^{++}	0.09 ± 0.20	0.0 ± 0.0

Data are means ± SD. NC, negative control (acetone 2%); MMS, methyl methanesulfonate (10 µg/mL, positive control); pre-treatment, 24 h Kavain + 24 h MMS; simultaneous treatment, 24 h distilled water + 24 h combined Kavain and MMS; post-treatment, 24 h MMS + 24 h Kavain. Cm, C-metaphase; CL, chromosome loss; NB, nuclear bud; MN, micronuclei; CB, chromosome bridge; CF, chromosome fragment. *Significant difference for the Kavain compared to NC. +Significant difference for pre, simultaneous and post-treatment compared to MMS. Significant by Kruskal-Wallis test with a posteriori Student-Newman-Keuls test (* $p < 0.05$; **/++ $p < 0.01$). Data are for 5,000 cells/treatment. The acetone 2% was used as a negative control, but how the results were statistical identical to solvent (distilled water), the data using water were omitted. The positive control I (MMS solubilized in distilled water) and positive control II (MMS solubilized in acetone 2%) also were statistical identical, the data using the positive control I were omitted.

fibers, chromosomal breaks and/or mitotic segregation of *A. cepa* cells (Bianchi et al. 2016, Pereira et al. 2020). Similar results were reported by Silva et al. (2021), who evidenced the non-mutagenic and/or recombinogenic effect (32, 64 and 128 µg/mL) in *D. melanogaster*. The antioxidant potential of kavain (Wruck et al. 2008, Sing et al. 2018) probably prevented the damage to the genetic material, and thus contributed to the reduction of chromosomal changes in the present study.

Regarding the cytoprotective effect on *A. cepa* cells, there was a significant increase in MI by kavain at the highest pre-treatment concentration (128 µg/mL) and at the two lowest post-treatment concentrations (32 and

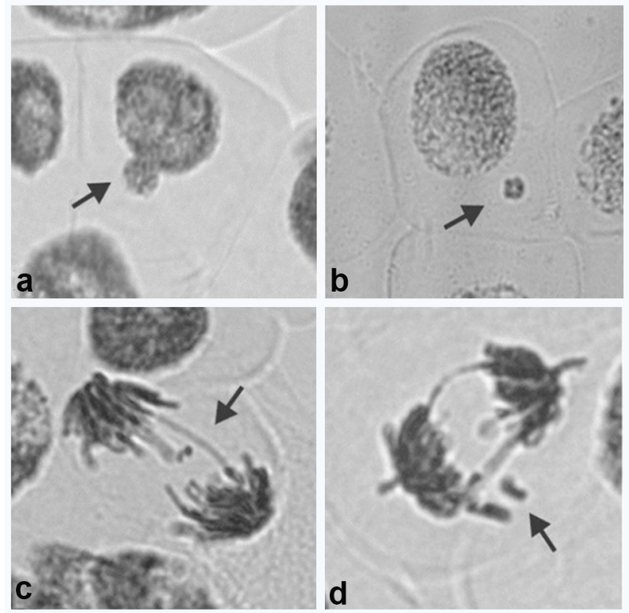

Figure 1. Chromosomal alterations observed by the analysis of meristematic cells from *Allium cepa* roots. a) nuclear bud (arrow); b) micronucleus (arrow); c) chromosomal bridge (arrow); d) chromosomal breaks (arrow).

64 µg/mL) compared to MMS (Table I). While in the other concentrations of the pre- (32 and 64 µg/mL), the simultaneous (32, 64 and 128 µg/mL) and the post-treatment (128 µg/mL), even though no significant difference was detected in relation to MMS, there was an increase in MI compared to MMS, except for the intermediate concentration of the simultaneous, contributing to a "trend" of the cytoprotective effect. Thus, the cytoprotective effect indicated the possible interaction of kavain in a direct and/or indirect way with MMS, decreasing and/or neutralizing its cytotoxic action.

Alkylating agents, such as MMS, reduced glutathione-S-transferase (GST) in mammalian cells, cause oxidative stress (Liu et al. 1996). MMS likely reduced GST in the meristematic cells of *A. cepa*, which normally contain high levels of GST (Hossain et al. 2007). Loss of GST decreases the antioxidant defense of cells, which results in accumulation of reactive oxygen species (ROS). ROS may increase the risk of DNA damage, including cell division with unrepaired or misrepaired damage, which cause mutations (Kehrer & Klotz 2015). In addition, ROS may be associated with decreased MI in meristematic cells of *A. cepa* (Bianchi et al. 2016), because they cause oxidation of lipids, alterations in membrane fluidity and DNA damage. The reduced MI in response to DNA damage mainly during the G1 and G2 phases occurs to allow the cells to repair damage before replicating their DNA and starting mitosis (Feng et al. 2010). Kavain may have neutralized the free radicals resulting from the action of MMS, since the isolate has antioxidant activity (Singh et al. 2018).

Kavain promoted the protective effect in all concentrations of the pre- (81.49 to 94.76%) and simultaneous (85.53 to 100.53%) and in the highest concentration of the post-treatment (88.33%) against mutagenic action of MMS (Table I). In the pre-treatment (demutagenic action), the isolated compound may have directly interacted with MMS in the intracellular

environment, preventing its mutagenic action in *A. cepa* cells (Felicidade et al. 2014). For the simultaneous treatment, the reduction in cell damage can be a result of both the demutagenic and bioantimutagenic action (Mauro et al. 2014) by the tested bioactive. In the post-treatment, kavain also promoted the reduction of damages at the highest concentration (128 µg/mL) by the bioantimutagenic action, which acts in DNA repair mechanisms, inducing the reversal of the mutagenic effect and preventing the fixation of mutations (Dametto et al. 2017, Fedel-Miyasato et al. 2014).

As the percentage of damage reduction (%DR) in the present study was higher for simultaneous treatment, the mechanism of action would be both demutagenic and bioantimutagenic. However, the protective action of the pre- was greater than the post-treatment, which shows that the major mechanism of action of kavain was demutagenic. The protective effect of kavain has also been observed in mice in pre-treatment against the toxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is a neurotoxin that causes symptoms of Parkinson's disease by destroying dopaminergic neurons in rat nerve cells (Schmidt & Ferger 2001). Wruck et al. (2008) observed the neuroprotective effect of kavain in the pre-treatment against the accumulation of β-amyloid plaques, which can block the signaling between nerve cells in the synapses.

Silva et al. (2021) administered only kavain (32, 64 and 128 µg/mL) simultaneously with Doxorubicin (DXR) in drosophilas and showed the protective effect only at the concentration of 32 µg/mL (75.94%) and 64 µg/mL (53.66%) at ST (standard) and HB (High bioactivation) crossings, respectively. This result demonstrates the sensitivity of the *A. cepa* test system, more able to detect antimutagenic events, as predicted by Leme & Marin-Morales (2009). On the other hand, the higher efficiency of the *A. cepa* test compared to drosophila can be explained by the metabolism of the organisms. The cytochrome P450 enzyme is responsible for 50% of the metabolism of therapeutic agents, and the comparison of the presence of this enzyme complex leads to the conclusion that plants have a lower concentration compared to mammals and insects (Rocha et al. 2016, Leme & Marin-Morales 2009). Thus, kavain, by inhibiting several cytochrome P450 isoforms (Mathews et al. 2002, Zou et al. 2004) may have decreased DXR metabolism and resulted in the least protective effect observed by Silva et al. (2021).

MMS was used in the present study as an inducer of DNA damage in the *A. cepa* assay. There are two main mechanisms by which this compound can act. The first is its known capacity for alkylation and methylation, which can cause breaks in the double strand of DNA and inhibit the replication fork (Chatterjee & Walker 2017). The second is its induction of high levels of oxidative stress, which can lead to apoptosis, cell death and DNA damage (Jiang et al. 2016). Studies demonstrate the ability to deplete Glutathione-S-transferase (Liu et al. 1996) and Glutathione (Siddique et al. 2019) of MMS, which impairs cellular antioxidant defenses and leads to the accumulation of free radicals generated as by-products from normal cell function (Raza 2011). Probably, kavain acted by neutralizing the action of MMS by the two mechanisms mentioned, once the direct mutagenic action of MMS was reduced in the protocols, mainly in the pre- and simultaneous and at the highest concentration of post. In addition, kavain may also have acted by the second mechanism mentioned, in which the isolated molecule would have neutralized the free radicals resulting from the action of MMS, since the isolate has antioxidant activity (Singh et al. 2018).

The protective effect of kavain in the pre- and simultaneous is related to the significant

reduction in micronuclei (MN), nuclear buds (NB) and chromosomal losses (CL) in all concentrations, except for NB at 64 µg/mL in the pre-, in relation to MMS (Table II). This result reinforces that kavain when interacting with MMS, probably prevented the aneugenic and/or clastogenic action of MMS in provoking the mentioned chromosomal alterations. A similar result for NB and CL was also found in all post-treatment concentrations. However, only at the highest concentration (128 µg/mL), there was a significant reduction in MN, which contributed to the highest %RD.

The results of this study demonstrated that kavain did not interfere with the progression of the cell cycle (mitotic index) and did not result in significant chromosomal changes caused by aneugenic and/or clastogenic mechanisms, indicating the absence of cytotoxicity and mutagenicity in *A. cepa*. In addition, kavain demonstrated an important chemopreventive activity, which is indirectly related to prevention and/or treatment of diseases, such as cancer. However, further studies are required to elucidate the biochemical mechanisms of interaction between kavain and the agent that induces DNA damage.

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