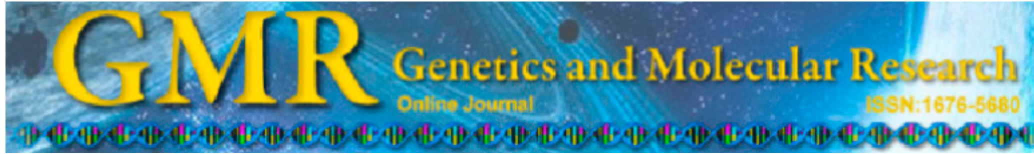

Evaluation of the antimutagenic activity and mode of action of the fructooligosaccharide inulin in the meristematic cells of *Allium cepa* culture

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ABSTRACT. This study evaluated the mutagenicity and antimutagenicity of inulin in a chromosomal aberration assay in cultures of the meristematic cells of *Allium cepa*. The treatments evaluated were as follows: negative control - seed germination in distilled water; positive control - aqueous solution of methyl methanesulfonate (10 μg/mL MMS); mutagenicity - aqueous solutions of inulin (0.015, 0.15, and 1.50 μg/mL); and antimutagenicity - associations between MMS and the different inulin concentrations. The antimutagenicity protocols established were pre-treatment, simultaneous simple, simultaneous with pre-incubation, and post-treatment. The damage reduction percentage (DR%) was 43.56, 27.77, and 55.92% for the pre-treatment; -31.11, 18.51, and 7.03% for the simultaneous simple; 30.43, 19.12, and 21.11% for the simultaneous with pre-incubation; and 64.07, 42.96, and 53.70% for the post-treatment. The results indicated that the most effective treatment for inhibiting damages caused by MMS was the post-treatment, which was followed by the pre-treatment, suggesting activity by bioantimutagenesis and desmutagenesis. The *Allium cepa* assay was demonstrated to be a good screening test for this type of activity because it is easy to perform, has a low cost, and shows DR% that is comparable to that reported studies that evaluated the prevention of DNA damage in mammals by inulin.

Key words: Inulin; Fiber; Antimutagenic activity

INTRODUCTION

The 20th century is characterized by the increase of chronic and degenerative diseases with multifactorial pathologies. Cancer is one these diseases. It develops, among other reasons, because of cellular abnormalities that correlate with alterations in the expression of proto-oncogenes and tumor suppressor genes; these mutations are the main events leading to the activation of a proto-oncogene or the inactivity of a tumor suppressor (Pienta et al., 1989; Bos and van Kreijl, 1992; Pitot, 1993; Kerr et al., 1994; Pool-Zobel et al., 2002).

These facts increase the concern about the recent exposure of humans to chemical agents from anthropogenic actions, such as industrial, agricultural, urban, and domestic residues. These, among other agents, can lead to the appearance of cellular abnormalities (Rigonato et al., 2004). Therefore, we verified the need to develop preventive strategies by means of studies that identify substances with chemoprotective potential for the genetic material (De Flora and Ferguson, 2005).

Fructooligosaccharide inulin is among the compounds with antimutagenic potential; it is a linear polydisperse carbohydrate and is essentially composed of fructil-fructose β (2→1) bonds (Hauly and Moscatto, 2002). Inulin-type fructans are present in several vegetables such as onion, banana, garlic, asparagus, and chicory. However, the only unit used at an industrial scale, so far, is extracted from the root of *Cichorium intybus*, which is popularly known as chicory (Van Loo et al., 1999; Roberfroid, 2005).

Inulin is widely studied for its nutritional characteristics; it is classified as a prebiotic fiber and functional food like wheat (Pesarini et al., 2013) and β-glucan (Oliveira et al.,

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2013a). Both classes stand out because they present different chemoprotective degrees and anticancer activities (Ferguson, 1994).

The anticarcinogenic activity of this fructan was verified by studies related to the development of colorectal carcinomas and their prevention by inulin supplementation (Pool-Zobel et al., 2002). Hughes and Rowland (2001) affirmed that inulin modulates the antitumorigenic capacity by apoptosis. Reddy et al. (1997), Bolognani et al. (2001), and Verghese et al. (2002) suggested that inulin has the capacity to suppress the induction of aberrant crypt outbreaks. Despite these descriptions, only one study evaluated the antigenotoxic and antimutagenic effects of inulin using comet and micronucleus assays in an attempt to better understand the mode of action of this compound in the prevention of DNA damage (Mauro et al., 2013).

Despite this information, the modes of antimutagenic action of inulin and its interaction with DNA have not been well described (Taper and Roberfroid, 1999). The resolution of this issue can increase the scope of fructan use as a functional food to prevent DNA lesions and consequently prevent cancer.

The use of plants, especially of the *Allium* genus, as a test system for the toxicological evaluation is validated by several international healthcare and environmental agencies, such as the United Nations Environmental Program, World Health Organization, and United States Environmental Protection Agency. This assay is widely popular because it can be used to efficiently assess chromosomal aberrations in cytogenetic tests (Fernandes et al., 2007). Therefore, this study aimed to evaluate the mutagenicity and antimutagenicity of inulin in assays with meristematic cells of *Allium cepa* and to describe the antimutagenic mode of action of this fructooligosaccharide.

MATERIAL AND METHODS

Chemical agents

Methyl methanesulfonate (*MMS*)

To induce DNA damage, we used 10 μg/mL MMS. This compound was chosen as a damage-inducing agent because it acts as an alkylating agent, adding alkyl groups in several positions of the DNA bases. This agent is capable of inducing a variety of lesions including adducts, cross-links, and breaks in the DNA chain. These alterations can be expressed as chromosomal aberrations and can be evaluated, for example, by the *A. cepa* assay.

Inulin

Inulin was donated by ORAFTI. Inulin was used at concentrations of 0.015, 0.15, and 1.50 μg/mL.

Experimental design

Commercial seeds of *A. cepa* (Isla® - Brazil) were used in this study. The seeds were placed on culture dishes to germinate at room temperature, covered with paper filter, and soaked with 3 mL distilled water or solution. Then, they were submitted to different treatments and pro-

tocols to evaluate mutagenicity and antimutagenicity. The seeds were cultivated for 120 h in the following treatments to evaluate the mutagenicity and antimutagenicity of inulin, which are described in Figure 1. I) Control (negative control) - seeds were cultivated for 120 h in distilled water. II) MMS (positive control) - seeds were cultivated in distilled water (3 mL) for 24 h and then transferred to culture dishes containing 3 mL 10 µg/mL MMS aqueous solution for 96 h. III) Inulin - seeds were cultivated in distilled water for 24 h and then germinated in aqueous solutions of inulin (0.015, 0.15, and 1.50 µg/mL) for 96 h. IV) Pre-treatment - seeds were kept in distilled water 24 h, cultivated in an aqueous solution of inulin (3 mL) for 48 h, and transferred to a dish containing 3 mL MMS for 48 h. V) Simultaneous simple - seeds were cultivated in 3 mL distilled water for 72 h and transferred to a culture dish containing 3 mL aqueous solutions of MMS and inulin, added simultaneously, for 48 h. VI) Simultaneous with pre-incubation - seeds were primarily germinated in distilled water for 72 h; aqueous solutions of MMS and inulin were pre-incubated in an oven at 37°C for 1 h, and the seeds were germinated in 3 mL of this solution for 48 h. VII) Post-treatment - seeds were cultivated in 3 mL distilled water for 24 h and transferred to a dish containing 3 mL aqueous solution of MMS for 48 h; then, the seeds were transferred to a dish containing 3 mL aqueous extract of inulin for 48 h. Prior to transferring the seeds from one dish to the other, they were washed twice in distilled water to remove compounds from the treatments. All treatments were performed in triplicate.

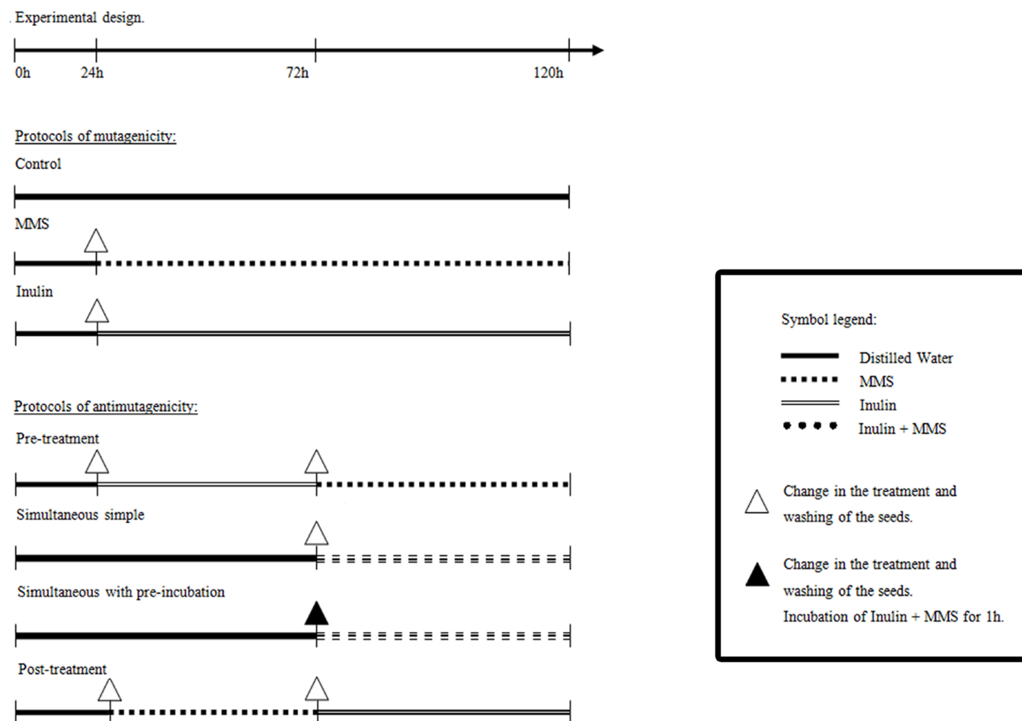

Figure 1. Treatments to evaluate the mutagenicity and antimutagenicity of inulin. Groups: Negative control = distilled water 96 h; MMS = aqueous solution of methyl methanesulfonate; pre-treatment (48-h inulin + 48-h MMS), simultaneous simple (48-h distilled water + 48 h of association of inulin and MMS), simultaneous with pre-incubation (48-h distilled water + 48-h MMS with inulin pre-incubated for 1 h at 37°C in the oven), post-treatment (48-h MMS + 48-h inulin).

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Slide preparation

According to the protocol by Fernandes et al. (2007), the roots were extracted at noon after 120 h and immersed in fixating solution (3:1 acetic acid:ethanol) for a minimum of 6 h. Later, the roots were subjected to acid hydrolysis to extract the genetic material (1 N HCl at 60°C for 6 min). Then, the DNA was stained in Schiff reagent for 2 h in the dark. The tips of the roots were cut with a scalpel to extract the meristematic region. The staining of the cellular cytoplasm was performed by adding a drop of 2% acetic carmine. Then, the material was covered by a small slide, and the meristems suffered light crushing with tweezers. To make the permanent slides, the entire slide was dipped in liquid nitrogen to remove the small slide. Later, with the aid of a synthetic resin (Permount®), a new small slide was placed over the biological material.

Microscopic and statistical analysis

A total of 15,000 cells/treatment (500 cells/slide) were analyzed by light microscopy with 40X magnification. During the analyses, the cells were subdivided into interphase, prophase, anaphase, and telophase; the number of chromosome alterations and the presence of a micronucleus were quantified in each phase.

To obtain the mitotic index (MI), the number of cells in division (interphase, prophase, anaphase, and telophase) was divided by the total number of cells analyzed.

$$MI = \frac{\text{Cells in division}}{\text{Total number of cells analyzed}}$$

The damage reduction percentage of the mitotic index (DRMI%), suggested by Fiskesjö (1993a), was calculated using the following formula: MI negative control - MI protocols = (result x 100) / MI negative control.

The damage reduction percentage (DR%), suggested by Waters et al. (1990), was obtained using the following calculation:

$$DR\% = \left[\frac{\text{Mean of positive control} - \text{Mean of associated group}}{\text{Mean of positive control} - \text{Mean of negative control}} \right] \times 100$$

The statistical analysis was performed by the chi-square test, and differences were considered to be statistically significant when $P < 0.05$.

RESULTS

The mutagenicity and antimutagenicity analysis of the fructooligosaccharide inulin indicated that the fructan inulin is not mutagenic at the concentrations tested (Table 1). The number of total aberrations in the control treatment was 2; that in the MMS treatment was 367; and that in the 0.015, 0.15, and 1.50 µg/mL inulin groups was 8, 6, and 4, respectively.

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Table 1. Distribution of chromosomal aberrations, MI, DRMI%, and DR% in *Allium cepa* treatments associated with inulin.

Treatment	DRMI%	MI	Chromosomal aberrations						Total	DR%
			MN	BRI	BRE	LO	SPR	MAN		
Mutagenicity										
Control	-	4.55	1	0	1	0	0	0	2	-
MMS	76.00	1.08[a*]	232	34	40	24	15	22	367[a*]	-
Inulin 0.015 µg/mL	34.00	3.01[a*]	6	2	0	0	0	0	8[a]	-
Inulin 0.15 µg/mL	35.50	2.98[a*]	4	0	0	2	0	0	6[a]	-
Inulin 1.50 µg/mL	36.00	2.90[a*]	4	0	0	0	0	0	4[a]	-
Antimutagenicity										
0.015 µg/mL										
Pre-treatment	50.54	2.25[b*]	121	25	41	0	17	4	208[b*]	43.56
Simultaneous simple	70.54	1.34[b]	222	165	64	21	8	1	481[b*]	-31.11
Simultaneous with pre-incubation	25.00	3.41[b*]	95	55	23	44	33	6	256[b*]	30.43
Post-treatment	14.50	3.89[b*]	89	10	18	14	1	0	132[b*]	64.07
0.15 µg/mL										
Pre-treatment	75.00	1.14[b]	116	22	62	23	33	9	265[b*]	27.77
Simultaneous simple	56.00	2.01[b*]	143	43	55	38	13	7	299[b*]	18.51
Simultaneous with pre-incubation	51.00	2.21[b*]	103	54	62	48	26	3	296[b*]	19.12
Post-treatment	23.95	3.46[b*]	88	29	27	49	16	0	209[b*]	42.96
1.50 µg/mL										
Pre-treatment	32.00	3.10[b*]	64	24	38	19	12	4	161[b*]	55.92
Simultaneous simple	64.00	1.64[b]	255	18	24	17	24	3	341[b*]	7.03
Simultaneous with pre-incubation	54.00	2.09[b*]	169	37	29	34	18	2	289[b*]	21.11
Post-treatment	6.00	4.27[b*]	77	23	35	24	9	2	170[b*]	53.70

DRMI% = damage reduction percentages of the mitotic index; MI = mitotic index; DR% = damage reduction percentages; MN = micronucleus; BRI = bridge; BRE = break; LO = loss; SPR = sprout; MAN = multipolar anaphase. MMS = methyl methanesulfonate. Experimental groups: control = distilled water 96 h; MMS = aqueous solution of MMS; pre-treatment (48-h inulin + 48 h MMS), simultaneous simple (48-h distilled water + 48 h association of inulin and MMS), simultaneous with pre-incubation (48-h distilled water + 48-h MMS with inulin pre-incubated for 1 h at 37ºC in the oven), post-treatment (48 h MMS + 48 h inulin). [a]Values compared to the control group. [b]Values compared to the MMS group. *Statistically significant differences (chi-square test, $P < 0.05$).

The protocols that were used to evaluate the antimutagenic effects of inulin provided a different DR% for different concentrations. The DR% of the pre-treatment were 43.56, 27.77, and 55.92%; those of the simultaneous simple treatment were -31.11, 18.51, and 7.03%; those of the simultaneous treatment with pre-incubation were 30.43, 19.12, and 21.11%; and those of the post-treatment were 64.07, 42.96, and 53.70% with 0.015, 0.15, and 1.50 µg/mL inulin, respectively.

The MI for the control was 4.55, and that for the MMS group was 1.08. For the inulin groups, we determined MI values of 3.01, 2.98, and 2.90 for the 0.015, 0.15 and 1.50 µg/mL concentrations, respectively. The DRMI%s were 76.00, 34.00, 35.50, and 36.00% for the MMS and the 0.015, 0.15 and 1.50 µg/mL inulin groups, respectively.

In the evaluation of antimutagenicity, we verified that the MI was always lower and statistically significant when compared to the control because of the presence of MMS. However, the MI was improved using the pre-treatment, simultaneous with pre-incubation and post-treatment protocols with 0.015 µg/mL inulin, and the MIs were 2.25, 3.41, and 3.89, respectively. On the other hand, the simultaneous simple protocol did not have an improved MI compared to that with MMS. The DRMI% varied from 14.50 to 70.54%.

With 0.15 µg/mL inulin, the only protocol that did not result in a statistically significant improvement of the MI was the pre-treatment. In this case, there was a reduction of

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75.00%, and the MI was 1.14. The MIs of the simultaneous simple, simultaneous with pre-incubation, and post-treatment protocols were 2.01, 2.21, and 3.46. The DRMI% were 56.00, 51.00, and 23.95% with 0.015, 0.15, and 1.50 μg/mL inulin, respectively. Therefore, inulin favors cell division when facing DNA damage from MMS.

With the highest inulin concentration tested, 1.50 μg/mL, the simultaneous simple protocol did not improve the MI and its DR%, which were 1.64 and 64.00%, respectively. However, inulin preserved the mitotic indexes in the pre-treatment, simultaneous with pre-incubation, and post-treatment, which varied from 2.09 to 4.27, while the DRMI% varied from 6.00 to 54.00%. Interestingly, the most frequent alterations were the presence of a micronucleus, bridges, and breaks.

DISCUSSION

Historically, plants are used as biomarker organisms in mutagenesis studies. The *A. cepa* assay has well-defined genetic markers, which include chromosomal aberrations (Grant, 1994). Rank and Nielsen (1997) reported that if a chemical agent is capable of causing chromosomal damage in plants, this capacity can be correlated to mutagenic events in mammals. The *A. cepa* assay is suggested as a method to screen mutagenic substances. In addition, the same tests that are used to evaluate mutagenicity can be used to evaluate antimutagenicity. However, for this to happen, it is necessary to combine two compounds, one that is widely known to be mutagenic and another with antimutagenic potential, to make adequate comparisons to evaluate the real efficacy of the later.

Mutations in genes that control essential cellular activities like proliferation, differentiation, and apoptosis can lead to the development of neoplasms (Pienta et al., 1989; Bos and van Kreijl, 1992; Pitot, 1993; Kerr et al., 1994; Pool-Zobel et al., 2002). Thus, finding compounds that decrease these and other frequencies of mutations can be a great gain in maintaining the integrity of the cellular genome and therefore possibly preventing diseases like cancer.

Constituents of diets can suppress genotoxic and mutagenic events and even damages caused by xenobiotics through intra- or extracellular activity, which is known as bioantimutagenic or desmutagenic modes of action. These modes of action can reduce the frequency of mutations (Hayatsu et al., 1988; Ferguson, 1994; el Hamss and Idaomar, 2002;) in a population or a determined group of cells. This would be beneficial because mutations generally determine the loss of genetic stability and increase the adverse phenotypes that are correlated with diseases.

The fructan analyzed in this study is a fiber that constitutes human diets. Its antitumor activity was evaluated in several studies (Reddy et al., 1997; Bolognani et al., 2001; Hughes and Rowland, 2001; Pool-Zobel et al., 2002; Verghese et al., 2002). Therefore, it is important to evaluate the antimutagenic potential of this compound and evaluate the modes of action of this fiber, which would indirectly prevent cancer.

We evaluated the mutagenicity of inulin and noted that it did not show mutagenic activity. This fact can also be corroborated by the results of a study by Mauro et al. (2013), where the authors used *in vivo* protocols to demonstrate that the tested compound did not present mutagenic activity when it was evaluated by the micronucleus assay in peripheral blood. This same substance was not correlated with the development of aberrant crypt outbreaks, which is another method of indirect measurement of mutations and tumor development. However, these authors demonstrated that the 50 mg/kg dose is related to the occurrence of DNA dam-

age when evaluated by the comet assay. In this assay, the animals that were treated with inulin had 47.81 times more comets than the control animals. Although, these genotoxic damages were not repaired, an increase in micronuclei was not confirmed. We did not find other correlated studies with similar discussions. However, Mauro et al. (2013) stated that the dose used in the experiment is within that recommended for human use.

According to Roberfroid (2005), the recommended consumption of inulin in Europe is 3-11 g. Making a correlation between the quantity of inulin ingested daily and the weight of the adult population in mg/kg, the consumption should be approximately 42.86-157.14 $mg \cdot kg^{-1} \cdot day^{-1}$ and that in the United States of America should be 14.28-57.14 $mg \cdot kg^{-1} \cdot day^{-1}$. Thus, the dose tested by Mauro et al. (2013) is within the recommended and used doses by the human population, where no genotoxic activity of inulin is expected. Therefore, this finding can be a warning for the necessity of further and new studies on the genotoxicity of this compound.

Despite the knowledge of the non-mutagenic activity of inulin that could aid its use in human food, it is necessary to know its true benefits with respect to DNA damage so that its properties as a functional food are properly evaluated. For such, the literature suggests several protocols to evaluate antimutagenicity, which attempt to elucidate the possible modes of action of natural compounds; these methods also can be used for this fructan. This study used pre-treatment, simultaneous simple, simultaneous with pre-incubation, and post-treatment protocols to evaluate the antimutagenicity of inulin. The simultaneous simple protocol indicated the desmutagenic and bioantimutagenic activity, the simultaneous with pre-incubation protocol indicated the desmutagenic activity, and the pre- and post-treatment protocols indicated preferably a biomutagenic activity (Ferguson, 1994; Flagg et al., 1995; Antunes and Araújo, 2000; De Flora and Ferguson, 2005; Oliveira et al., 2006, 2007, 2009, 2013b; da Silva et al., 2013).

The simultaneous simple treatment presented the lowest DR% in the three different concentrations when compared to the other protocols. The 0.015 µg/mL concentration presented a negative DR% (-31.11%), suggesting the absence of chemoprotective activity, and it possibly increased the damage caused by MMS. However, no reports were found in the pertinent literature that could help in the comprehension of these data. From the other concentrations in this protocol (0.15 and 1.50 µg/mL), inulin has low efficiency desmutagenesis and/or bioantimutagenesis. Therefore, it can be assumed that a possible connection between the MMS and the inulin would be responsible for the inactivity of the MMS, and it would not be efficient in the extra- nor the intracellular medium. Even if the inulin penetrated the cells, it was not efficient in modulating their repair system.

The simultaneous group with pre-incubation presented a DR% that varied from 19.12 to 30.43%. Despite the low DR%, results suggested activity by desmutagenesis for the three tested inulin concentrations. In addition, this fact suggests a low capacity of the fiber in absorbing the MMS when these two compounds were pre-incubated at 37°C. However, this small adsorption demonstrated improvement in the inulin's chemoprevention.

In the pre-treatment protocol, there was an important gain in the inulin's chemopreventive activity, and the DR% were 43.56, 27.77, and 55.92% with 0.015, 0.15, and 1.50 µg/mL inulin, respectively. This suggests that inulin can modulate repair enzymes of the cellular DNA as suggested by Roland et al. (1993, 1994, 1995, 1996) and Mauro et al. (2013). Therefore, we supposed that the inulin entered the meristematic cells of *A. cepa* and modulated the repair system, making it more efficient when preventing or repairing the damage caused by the alkylating action of MMS.

When the post-treatment protocol was evaluated, we observed the highest DR%, which varied from 42.96 to 64.07%. This protocol indicated a bioantimutagenic mode of action, which reinforced the data from the pre-treatment protocol and the remarks made by other authors in other biological assays (Roland et al., 1993, 1994, 1995, 1996; Mauro et al., 2013). This protocol suggests and confirms the action of inulin modulating the repair system. Through this action, it can modulate the enzymes in plant cells to more efficiently repair the genetic damage already caused by the previous exposure to MMS.

According to these data, the preferential chemopreventive mode of action for inulin is the bioantimutagenic activity. The same consideration was made by Mauro et al. (2013), who observed that inulin shows the capacity to act by desmutagenesis and bioantimutagenesis. However, the last mechanism of action seems to prevail independently in models that evaluate inulin activity in mammals or plants. This fact suggests that the *A. cepa* assay can be used to screen antimutagenic substances with potential applications in the prevention of mutation in mammals.

Despite the possibility of using the *A. cepa* assay to determine the antimutagenic effect of inulin and to indicate its antimutagenic mechanism of action, inulin has a much more accentuated chemopreventive action in mammals. This possibly can be because the metabolic capacity of mammals is much larger than that of the *A. cepa* system.

The DR% in the mutagenicity assay in mammals varied from 47.25 to 141.75%, while that in the plant assay varied from -31.11 to 64.07%. This difference might be explained because, according to some authors, inulin plays its antimutagenic and anticarcinogenic role when it suffers metabolization in the animals' intestines. In this way, the metabolites of this fiber might be more efficient in stopping the direct and indirect mechanism of procarcinogens (Roberfroid, 2005). If this fact is true, an alternative to better assess the *A. cepa* assay to screen antimutagenic and/or anticarcinogenic substances, such as probiotics, would be to use fraction S9 along with the culture system or to use probiotics that were fermented by *Bifidobacterium*. Therefore, we would have a result where the DR% would be closer to that observed by Mauro et al. (2013) because, in this case, the inulin was metabolized in the intestines of mice.

Generally, we observed that the DR% described above was not significant facing the already studied anticancer capacity of inulin. Maybe this fact is enlightened by a property that is characteristic of food fibers, their fermentation in the intestinal colon (Reddy, 1999; Cherbut, 2002). This experiment does not simulate the fermentation; thus, the metabolites produced by fermentation (Cherbut, 2002; Roberfroid, 2005) did not act on the analyzed roots, decreasing the competency of inulin to reduce the frequency of mutations in the study described here.

However, the reductions in frequencies of mutations studied reflect the possible mechanisms of this fiber in the activation of antioxidant and detoxifying enzymes, such as glutathione S-transferase and glucuronosyltransferase (Roland et al., 1993, 1994, 1995, 1996).

Fiskesjö (1993a) affirmed that the MI of apical meristems of *A. cepa* facing the chemical substances is considered normal when there is inhibition of cellular division up to 36%. The same author still reports that when the MI is reduced in values under 50%, the analyzed substance is toxic to the meristematic cells. These facts suggest that inulin is not toxic to the meristematic culture. Furthermore, its association with MMS improves the MI, indicating that this fiber has the capacity to modulate events to decrease the toxicity of this alkylating agent.

As mentioned before, the *A. cepa* assay is an easy and practical method of evaluating mutagens, but it can also be used to determine the toxicity caused by chemical treatments. This event is based on the principle that the growth of roots is inhibited when they are exposed to

toxic substances (Fiskesjö, 1993a,b, 1994; Rigonato et al., 2004). According to the reference values of Fiskesjö (1993a), the MIs that were calculated in the mutagenicity protocols show that the inulin does not have genotoxic activity in any of the three concentrations. The DRMI% were 34.00, 35.50, and 36.00% for 0.015, 0.15, and 1.50 µg/mL, respectively. This fact suggests that the gradual increase of this fiber can decrease the meristematic MI and cause toxicity.

In relation to the protocols of antimutagenicity, the groups that demonstrated the lowest MIs and highest DRMI%s (more than 50%) were: pre-treatment at 0.015 and 0.15 µg/mL, simultaneous simple at the three different concentrations, and simultaneous with pre-incubation at 0.015 and 1.50 µg/mL. This conveys that these protocols and concentrations were not efficient in reducing toxicity caused by the damage-inducing agent MMS. However, the association of inulin shows that it is capable of partially reversing the toxicity scenario set by the treatment of cultures with this alkylating agent. On the other hand, the protocols of pre-treatment at 1.50 µg/mL, simultaneous with pre-incubation at 0.015 µg/mL, and all the concentrations of the post-treatment had high values of MIs and small DRMI%s (values lower than 36%), suggesting that the addition of inulin in the system of cellular cultivation significantly alters the toxicity caused by MMS.

A correlation was also seen between the protocols and concentrations in which the inulin did not act as an effective antimutagenic agent. In the same way, the groups with an effective antimutagenic mechanism are associated with high MIs, and the DRMI%s are below the reference values established by Fiskesjö (1993a). Therefore, it can be inferred that the groups without success as an antimutagenic agent also had no antigenotoxic activity. However, the antimutagenic groups with high antimutagenic activity had also high antigenotoxic activity.

Reports state that the genotoxic events can lead to a mutagenic event if there is no repair of the genetic material (Salvadori et al., 2003; Oliveira et al., 2006). The results obtained in this experiment corroborate with those in previously published reports.

In a final analysis of the results obtained, we suggest that inulin acts preferably by bioantimutagenesis because the highest DRMI%s were present in the pre- and post-treatment protocols. It is also important to note the fact that the groups, with reductions in their mutational frequencies, maintained the MI, which means that the genotoxic effect of MMS was decreased. Therefore, it can be inferred that the genotoxic damages, when repaired, decrease the frequency of mutations in meristematic cells of *A. cepa* when exposed to the fructooligosaccharide inulin. It is also worth mentioning that the correlation between the data obtained in this study corroborates the results already described in the literature. Further studies are needed so that the interaction between inulin and DNA can be better described and so the mechanism of action can be confirmed in test systems for mammals, especially *in vitro*. Therefore, taking into account data from Roland et al. (1993, 1994, 1995, 1996), Roberfroid (2005), Mauro et al. (2013), and the results presented in this study, we suggest that inulin, when consumed in the recommended concentrations, can be used as an adjuvant in chemotherapies, acting as an antimutagenic agent or even as a functional food that is capable of preventing the occurrence of mutations related to the origin of neoplasm.

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