

Study of the modulator effect of oil chia (*Salvia hispanica* L.) associated with benzo(a)pyrene and doxorubicin hydrochloride

Estudo do efeito modulador do óleo de chia (*Salvia hispanica* L.) associada benzo(a)pireno e cloridrato de doxorubicina

Estudio del efecto modulador de aceite de chía (*Salvia hispanica* L.) benzo(a)pireno y doxorubicina cloridrato

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Abstract

Chia (*Salvia hispanica* L.) is a Mexican plant belonging to the *Lamiaceae* family, representing one of the four main seeds cultivated by pre-Columbian peoples, mainly the Aztecs who used it as a medicine and nutritional component. Currently, it has been rediscovered, they are rich in polyunsaturated fatty acids, with antioxidant activity and essential compounds for organism maintenance. Various benefits are attributed to this plant including the reduction of cardiovascular problems, psoriasis, depression, Alzheimer's, diabetes, arthritis and cancer. Considering the widespread use of chia oil (OC) in today's society, it was justified to investigate the modulating effect of this oil when combined with benzo(a)pyrene and doxorubicin hydrochloride, using the SMART (Somatic Mutation and Recombination Test), a test able to detect mutation and somatic recombination activities, with loss of heterozygosity, which can be observed in the forms of different trichomes present in the wings of *Drosophila melanogaster*. The results obtained indicate that chia oil reduced the frequency of mutations by 97% in the number of spots induced by benzo(a)pyrene (BaP) and doxorubicin hydrochloride (DRX), showing an antigenotoxic effect.

Keywords: Antioxidant; Mutation; Antigenotoxic effect; *Drosophila melanogaster*; Somatic Mutation and Recombination Test.

Resumo

Chia (*Salvia hispanica* L.) é uma planta mexicana pertencente à família *Lamiaceae*, representando uma das quatro principais sementes cultivadas por povos pré-colombianos, principalmente os Astecas que a usaram como um medicamento e componente nutricional. Atualmente, foi redescoberta, elas são ricas em ácidos graxos poli-insaturados, com atividade antioxidante e compostos essenciais para a manutenção do organismo. Vários benefícios são atribuídos a essa planta, incluindo a redução de problemas cardiovasculares, psoríase, depressão, Alzheimer, diabetes, artrite e câncer. Considerando o uso generalizado de óleo de chia (OC) na sociedade atual, justificou-se a investigar o efeito modulador deste óleo quando combinado com benzo(a)pireno e cloridrato de doxorubicina, utilizando o SMART (Teste de Mutação Somática e Recombinação), um teste capaz de detectar atividades de mutação e recombinação somática, com perda de heterozigose, que pode ser observada nas formas de diferentes tricomas presentes nas asas da *Drosophila melanogaster*. Os resultados obtidos indicaram que o óleo de Chia reduziu as frequências de mutações ocasionadas pelo benzo(a)pireno (BaP) e cloridrato de doxorubicina (DRX), apresentando assim efeito antigenotóxico.

Palavras-chave: Antioxidante; Mutação; Efeito antigenotóxico; *Drosophila melanogaster*; Teste de Mutação Somática e Recombinação.

Resumen

La chía (*Salvia hispanica* L.) es una planta mexicana perteneciente a la familia *Lamiaceae*, que representa una de las cuatro principales semillas cultivadas por los pueblos precolombinos, principalmente los Astecas que la utilizaban como medicina y componente nutricional. Actualmente, se ha recordado, los ellas son ricos en ácidos grasos poliinsaturados, con actividad antioxidante y compuestos esenciales para el mantenimiento del organismo. A esta planta se le atribuyen varios beneficios, incluyendo la reducción de problemas cardiovasculares, psoriasis, depresión, Alzheimer, diabetes, artritis y cáncer. Teniendo en cuenta el uso generalizado del aceite de chía (OC) en la sociedad actual, se justificó investigar el efecto modulador de este aceite cuando se combina con clorhidrato de benzo(a)pireno y doxorubicina, utilizando SMART (Mutación somática y prueba de recombinación), una prueba capaz de detectar la mutación somática y las actividades de recombinación, con pérdida de heterocigotos, que se pueden observar en las formas de diferentes hogares tricomas presentes en las alas del *Drosophila melanogaster*. Los resultados indicaron que el aceite de chía redujo las frecuencias de mutaciones causadas por benzo(a)pireno (BaP) y doxorubicina clorhidrato (DRX), presentando así efecto antígeno tóxico.

Palabras clave: Antioxidante; Mupción; Efecto antígeno tóxico; *Drosophila melanogaster*; Mutación somática y prueba de recombinación.

1. Introduction

Somatic cells are subject to mutation mechanisms, loss of heterozygosity and rearrangement of their genetic material, the accumulation of these changes can lead to the formation of modified cells that may not respond to proliferation control mechanisms, death and cell differentiation (Ling et al., 2010).

In multicellular organisms, mitosis occurs to promote the growth, regeneration of living tissue and the replacement of dead cells, and for unicellular, this process is directly related to the reproduction of the species and an accurate transfer of genetic information (Follain et al., 2017).

The cell division control mechanism regulates the growth of cells to prevent the improper expansion of the genetic material, which results in instability in the genome. Changes in cell behavior, as a result of various development stages of genes through genetic mutations, chromosomal losses and, genomic instability and epigenetic mechanisms, ultimately lead to carcinogenesis stages (Pierron, 2015).

In biological systems, the main source of free radical species comes from oxygen, which is known as reactive oxygen species (ROS), however, oxygen is also of essential importance in cell metabolism and energy production. The greatest damages to the genome are induced by the exogenous agents that can affect the DNA through hydrolysis reactions of purines and pyrimidines. These damages are induced by ROS and reactive nitrogen species (RNS) that promote the formation of casual replication defects generating incorrect sequencing, and even filament breaks (Zheng et al., 2014).

The production of reactive species is an integral part of human metabolism and is observed in several physiological conditions. The presence of ROS and RNS has important biological functions, such as phagocytosis, a phenomenon in which these species are produced to eliminate an aggressive agent. However, when in high concentration, the organism uses efficient antioxidant mechanisms to control and restore balance, so the cellular damage results basically from an attack of ROS and RNS on organic macromolecules, such as sugars, DNA, proteins and lipids (Dröge, 2002).

According to the National Cancer Institute, most cancer cases (between 80 to 90%) are correlated to the external environment, to which numerous risk factors are found. The external environment is generally inferred from land, water and air; social and cultural environment, habits and lifestyle, consumption environment, medicines and food, occupational environment, chemical and related industries. Many lifestyles or habits chosen by individuals can cause changes in the external environment, being able to cause many types of pathologies, one of which is cancer (INCA, 2019).

Food has an essential role in providing beneficial bioactive components to the body and on the other hand, it can also be a source of harmful substances such as organophosphate and organochlorine pesticides, mutagenic and/ or carcinogenic agents. As the studies considering xenobiotic residues and antinutritional factors advance, contemporary society seems to adapt their dietary habits including the rescue of ancient grains to increase a healthy and balanced lifestyle. The increased interest in

the study of chia seeds is due to their nutrition and health-promoting properties (Marcinek & Krejpcio, 2017). Chia seeds can achieve 25 to 38% fat content and is considered one of the richest botanical sources of omega-3 α -linolenic acid (18:3, ALA), up to 68% (Marcinek & Krejpcio, 2017; Cahill, 2003; Coelho & Salas-Mellado, 2014; Melo et al., 2019).

When compared to any known vegetable source, chia seeds stand out as an important source of proteins, dietary fibers, minerals and bioactive compounds such as tocopherols and phenolics (Melo et al., 2019), increasing their beneficial effects on human health. Due to the high quantity and quality of benefits of chia seeds, several studies have also analyzed the extraction process and commercialization as vegetable oil (Barros et al., 2008; Ixtaina, 2011; Sargi et al., 2013; Coelho & Salas-Mellado, 2014).

The polyunsaturated fatty acids (PUFA) of the omega family (ω -3, ω -6), are essential lipids for health and as they are not synthesized by the human body, it is necessary to provide them in the dietary basis rich in fish and oilseeds. The ω -3 ALA is metabolized by the body to docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and the ω -6 (linolenic acid - LA) is metabolized to arachidonic acid as the product (Guo et al., 2020). The high ingestion of ω -3 causes beneficial health effects due to its ability to modulate metabolic and cellular functions, producing actions in the anti-inflammatory alteration that eicosanoids participate in, altering the structure of the cellular membrane and in several pathways of normal and pathological cellular (Gazi et al., 2006).

The ω -3 has different mechanisms of action in neoplastic cells, such as modulation of inflammation, cell multiplication, apoptosis and metastasis, increase or decrease in the production of free radicals. Therefore, the ingestion of EPA and DHA is essential to inhibit carcinogenesis and prevent the growth of tumor cells, promoting the efficacy of chemotherapeutics (Guo et al., 2020). Dubois et al., (2007), classified the oils according to their FA profiles and included PUFA class seed oil (subclass α -linolenic + linolenic acid), emphasizing the importance of providing a good balance between the two essential fatty acids, since it provides all nutrients and compounds necessary for the proper body function and prevention of chronic diseases.

According to Ramos et al. (2019), OC composition showed 45.81% linolenic acid, 19.95% linoleic acid, 12.49% palmitic acid and 10.29% oleic acid. Linolenic acid appears as the major fatty acid in OC from different studies. (Marineli et al., 2015a; Marineli et al., 2015b; Ramos et al., 2019). DXR is a chemotherapeutic substance with numerous therapeutic applications in human neoplasm, for example, carcinomas, testicular and breast tumors (Chiuchetta & Castro-Prado, 2002). As the drug belongs to the family of anthracyclines agents, it produces side effects due to the cumulative doses in the individual organism causing pathologies such as cardiotoxicity and bone marrow problems (Kwok & Richardson, 2003).

DXR is a non-specific agent of the cell cycle, therefore, the drug acts on dividing cells and cells in the stationary phase, while the main cytotoxic action occurs in the S-phase of the cell cycle. The mechanisms of action in tumor cells as a result of DXR administration can be observed as the generation of free radicals causing damage to genetic material (DNA), lipid peroxidation, cross-linking with the DNA molecule, propensity to damage by inhibition of DNA topoisomerase II (Minotti et al., 2004).

The species of free radicals produced by DXR involve reactions with iron. the iron ions react with DXR by the process of oxy-reduction reactions, reducing Fe^{+3} to Fe^{+2} and the Fe-DXR complex causes the reduction of oxygen to hydrogen peroxide, promoting the formation of other reactive oxygen species (ROS). Considering some highlights of the literature which suggest that polyunsaturated fatty acids (α -linolenic acid) from OC could play as modulators of the cellular antioxidant defense system (Chiuchetta & Castro-Prado, 2002). In this work, the authors propose to assess the antimutagenic capacity of OC and to investigate its modulating effect when associated with BAP and DRX, using the SMART assay with wings of *Drosophila melanogaster*.

2. Material and Methods

2.1 Chia Oil (*Salvia hispanica* L.)

The oil was purchased in a commercial pharmacy in the form of oily 500 mg capsules - Herbarium®, and were used to evaluate mutagenic and antimutagenic effect. To prepare stock solutions in different concentrations were used Milli-Q water, 1% of Tween-80 and 3% ethanol P.A for mutagenic (0.5, 1 and 2%) and antimutagenic (0.25, 0.5 and 1%) assays. The assays were associated with the DXR (0.2 mM) and (BaP) (2 mM) as positive control and the dilution solvent as negative control. All tests were realized in triplicate.

2.2 Somatic Mutation and Recombination Test (SMART)

The marker multiple wing hairs (*mwh*, 3-0.3) and flare-3 (*flr³*, 3-38.8) are at the tip and rough in the middle of the left arm of the arm of chromosomes 3, respectively. Two crosses were carried out to produce the experimental larval progeny: 1) Standard (ST) cross, *flr³/In(3LR)TM₃, ri p^p sep I (3)89Aa bx^{34e} e Bd^S* females crossed with *mwh* males; 2) High bioactivation (HB) cross, *ORR/ORR; flr³/In(3LR)TM₃, ri p^p sep I (3)89Aa bx^{34e} e Bd^S* females crossed with *mwh* males (Graf et al., 1983; Graf & Van Schaik, 1992).

From the two crosses, eggs were collected for 8h in culture bottles with an agar-agar base (4% w/v) topped with a thick layer of live baker's yeast supplemented with sucrose. The larvae were washed out of the bottles 72 ± 4 h were washed and collected with running water using a fine-mesh sieve and treated following two protocols (mutagenicity and antimutagenicity). In the mutagenicity protocol the larvae were transferred to glass vials containing 1.5 g of alternative growing medium (instant potato paste Yoki® - Yoki Alimentos S.A.) rehydrated with solutions containing different concentrations 90.5 e 2%) of OC.

For the evaluation of antimutagenicity, the larvae were transferred to flasks containing rehydrated alternative culture medium with a solution containing different concentrations (0.25, 0.5 and 1%) of OC, associated with doxorubicin hydrochloride (DXR) 0.2 mM, or associated with 2mM of BaP. For both protocols, positive control was used (0.2mM) and BaP (2mM) and as negative control the solvent (Milli-Q water 1%, Tween-80 and 3% ethanol P.A).

The emergent adults are carriers of the two types of genotypes: *mwh + / + flr³* (marked trans-heterozygote – MH) and *mwh + / + TM₃, Bd^S* (balanced heterozygous –BH) were collected and fixed in ethanol 70%. The wings were detached and mounted between Blades and laminules with Faure solution (30g Arabic gum, 50g chloral hydrate, 100mL of water and 20mL of glycerol) and analyzed for the occurrence of different types of mutant stains, in optical microscopes with a magnification of 400X.

2.3 Statistical analysis

The results observed in the groups treated with chia oil (OC) were statistically evaluated using the Conditional Binomial Test with significant level $\alpha = \beta = 0.05$ (Kastenbaum & Bowman 1970).

The frequencies of each fly spot type were compared with the respective negative controls, making it possible to characterize the results as positive (+), low-positive (f+), negative (-) and inconclusive (i), (Frei & Würigler, 1988).

To statistically evaluate the antimutagenic activity, the frequencies of each stain type: small single spots, large single spots, or twin spots and the total spots by flies of each treatment were compared to the pairs (positive control (DXR) or BaP isolated versus DXR or BaP associated as OC according to Kastenbaum & Bowman, (1970), with significant level $\alpha = \beta = 0.05$.

The calculation of the percentage of reduction (%R) was carried out from the mutation frequencies obtained from individuals who were treated with the samples associated with DXR or BaP, according to Abraham, (1994):

$$\% R = \text{mutation frequency DXR or BaP} - \text{mutation frequency of the OC associated the DXR or BaP} \times 100 / \text{mutation}$$

frequency DXR or BaP.

3. Results and Discussion

The table 1 presents the results obtained in the mutagenicity protocol, of the descendants of the crossings ST and HB treated with 0.5, 1 and 2% of OC. Comparing the frequency of clone formation by cell division in groups treated with OC, with the respective negative control, it is verified that the frequency of clone formation does not differ statistically from the negative control, at both crossings ST and HB. These results indicate that in the evaluated concentrations of OC there is no mutagenic activity. The results obtained with OC, are similar to those obtained by Anter et al. (2010) with the olive oil in SMART test at concentrations 1.25 at 12.5% in the descendants of crossings ST and HB, thus both oils have no mutagenic activity.

Table 2 presents the results obtained in the antimutagenicity protocol, of the descendants of the crossings ST and HB treated with OC in concentrations of 0.25, 0.5 and 1% associated with 0.2 mM of DXR. In the descendants of the ST cross, the individuals treated with DXR have shown a clone formation frequency of 14.65×10^{-5} , while they were treated with the presence of 0.25, 0.5 and 1% of OC and DXR, showed clone formation frequency of respectively 0.30, 0.41 and 0.82×10^{-5} .

OC showed a protective effect against the mutagenic damage of DXR, drastically reducing the number of stains observed in the treated groups, when compared with the positive DXR control, there is a reduction of 97% in concentrations 0.25 and 0.5 % and a reduction of 94% in concentrations of 1% of OC. A similar result was observed in the descendants of the HB crossing, whose reductions ranged from 77 to 97% (Table 2).

These data indicate that OC has antimutagenic activity, probably because it neutralizes free radicals generated by DXR. A similar result was obtained by Anter et al. (2010) when he evaluated the olive oil associated with hydrogen peroxide, in the SMART test. According to Fragiorgis et al. (2007) ascorbic acid reduced the effects of DXR, in the SMART assay, by scavenging free radicals generated by DXR. Also, Valadares et al. (2008) using the SMART assay, found that aqueous propolis extracts reduced the genotoxic effects caused by DXR, by neutralizing the free radicals generated. Table 3 presents the results of crosses of the ST and HB associated with OC and BaP. The ST descendants treated with BaP showed mutation frequencies from 0.30 to 0.31×10^{-5} and those from HB presented a frequency of 1.64×10^{-5} . The results indicate BaP is a pro-genotoxic substance, probably an indirectly acting mutagen that needs to undergo metabolism, oxidation, to bind covalently to nitrogen 7 in guanine and generate an adduct in DNA. As the ST strain has baseline levels of the cytochrome P-450 enzyme, BaP was not metabolized and therefore caused no DNA damage.

The results of BaP treatment in the HB descendants are by the literature, as this compound needs to be metabolized to become genotoxic. The biotransformation of polycyclic aromatic hydrocarbons (PAHs), including BaP, involves a series of enzymes that catalyze oxidation, reduction and hydrolysis reactions (mixed-function oxygen, cytochrome P-450, NADPH-cytochrome-c-reductase) and enzymes that catalyze conjugation reactions (sulfotransferase, epoxy dihydroxylase, glutathione-S-transferase and UDP-glucosyltransferase) (Valadares et al., 2008).

According to Naspinski et al. (2008), BaP metabolism by cytochrome CYP1A, a channel thus generated by active oxygen species (ROS), produces toxic metabolites such as BaP epoxides in the cellular environment, which can directly attack DNA. The adducts generated by BaP in DNA are responsible for chromosomal damage that lead to the formation of micronuclei (MN) (Vienneau & DeBoni, 1995; Sánchez et al., 2000).

Table 1 - Frequencies of mutant stains observed in wings of *D. melanogaster* descendants of standard (ST) and high bioactivation (HB) crossings treated with OC.

Crossing	Treatment concentration and	Individual numbers (N)	Stains per individual				Total stains Indiv.(N) (n)	Clone Formation Frequency/ 10 ⁻⁵ cells per division MSP ^d	
			MSP ^d (1-2 cells) ^b m = 2	stains (>2 cells) ^b m = 5	MG ^f m = 5	m = 2		(1-2 cells) ^b	(>2 cells) ^b
ST	NC	20	0.10 (02)	0.05 (01)	0.00 (00)	0.15 (03)	3	0.30	-
	OC 0,5%	20	0.15 (03) i	0.10 (02) i	0.00 (00) i	0.25 (05) i	5	0.51	0.21
	OC 1%	20	0.15 (03) i	0.05 (01) i	0.05 (01) i	0.25 (05) i	5	0.51	0.21
	OC 2%	20	0.15 (03) i	0.05 (01) i	0.00 (00) i	0.20 (04) i	4	0.40	0.10
HB	NC	20	0.40 (08)	0.10 (02)	0.00 (00)	0.50 (10)	10	1.02	-
	OC 0,5%	20	0.35 (07) i	0.00 (00) i	0.10 (02) i	0.45 (09) i	9	0.92	-0.1
	OC 1%	20	0.40 (08) i	0.00 (00) i	0.00 (00) i	0.40 (08) -	8	0.81	-0.21
	OC 2%	20	0.45 (09) i	0.00 (00) i	0.10 (02) i	0.55 (11) i	11	1.12	0.1

^a Statistical diagnosis according to Frei & Würzler (1988): +, positive; -, negative; i, inconclusive. m, multiplication factor for the evaluation of significantly negative results. Significance levels a = b = 0.05; ^b Including rare *flr*⁻³ simple spots; ^c Considering the *mwh* clones for single *mwh* spots and for twin spots; ^d Small simple stains; ^e Large simple spots; ^f Twin spots; ^g Sum of all observed mutant spots; (NC): Negative Control, Chia oil (OC). Source: Authors.

Table 2 – Frequencies of mutant spots observed in *D. melanogaster* wings descending from standard (ST) and high bioactivation (HB) crosses treated with OC associated with DRX.

Crossing	Treatment and concentration	Individual numbers (N)	Stains per individual diag. Statistic				Totalstains <i>mwh</i> ^c (n)	Frequency of clone formation /10 ⁻⁵ cells per division		
			MSP ^d (1-2 cells) ^b m = 2	stains (>2 cells) ^b m = 5	MG ^f m = 5	TM ^g m = 2		Observed	Correctedcontrol	Inhibition
ST	NC	20	0.10 (02)	0.05 (01)	0.00 (00)	0.15 (03)	3	0.30	-	-
	DXR	20	2.95 (59) +	2.25 (45)+	2.10 (42)+	7.30 (146)+	138	14.95	14.65	-
	OC 0,5% +DXR	20	0.25 (05) +	0.05 (01)+	0.00 (00)+	0.30 (06)+	6	0.61	0.30	97%
	OC 1% + DXR	20	0.20 (04) +	0.10 (02)+	0.05 (01)+	0.35 (07)+	7	0.71	0.41	97%
	OC 2% +DXR	20	0.45 (09) +	0.05 (01)+	0.05 (01)+	0.55 (11)+	11	1.12	0.82	94%
H	NC	20	0.40(08)	0.10 (02)	0.00 (00)	0.50 (10)	10	1.02	-	-
	DXR	20	2.95 (59) +	4.05 (81) +	1.05 (21) +	8.05 (161) +	158	16.49	15.47	-
	OC 0,5% +DXR	20	0.85 (17) +	0.85 (17) +	0.50 (10) +	2.20 (44) +	44	4.5	3.48	77%
	OC 1% + DXR	20	1.10(22) +	0.80 (16) +	0.30 (06) +	2.20 (44) +	44	4.5	3.48	77%
	OC 2% +DXR	20	0.30(06) +	0.15 (03) +	0.20 (04) +	0.65 (13) +	13	1.33	0.31	97%

^a Statistical diagnosis according to Frei & Würzler (1988): +, positive; -, negative; i, inconclusive. m, multiplication factor for the evaluation of significantly negative results. Significance levels a = b = 0.05; ^b Including rare *flr*³ simple spots; ^c Considering the *mwh* clones for single *mwh* spots and for twin spots; ^d Small simple stains; ^e Large simple spots; ^f Twin spots; ^g Sum of all observed mutant spots; (NC): Negative Control, Chia oil (OC). Source: Authors.

Table 3 – Frequencies of mutant spots observed in *D. melanogaster* wings descending from standard (ST) and high bioactivation (HB) crosses treated with OC associated with BaP.

Crossing	Treatment and concentration	Individual numbers (N)	Stains per individual diag. Statistic				Totalstains <i>mwh</i> ^c (n)	Frequency of clone formation /10 ⁻⁵ cells per division		
			MSP ^d (1-2 cells) ^b <i>m</i> = 2	stains (>2 cells) ^b <i>m</i> = 5	MG ^f <i>m</i> = 5	TM ^g <i>m</i> = 2		Observed	Corrected control	Inhibition or reduction
ST	NC	20	0.10 (02)	0.05 (01)	0.00 (00)	0.15 (03)	3	0.30	-	-
	BaP	20	0.20 (04) i	0.00 (00) i	0.10 (02) i	0.30 (06) i	6	0.61	0.31	-
	OC 0,25% + BaP	20	0.30 (06) i	0.20 (04) i	0.05 (01) i	0.55 (11) i	11	1.12	0.82	-
	OC 0,5% + BaP	20	0.30 (06) i	0.00 (00) i	0.00 (00) i	0.30 (06) i	6	0.61	0.31	-
	OC 1% +BaP	20	0.35 (07) i	0.05 (01) i	0.00 (00) i	0.40 (08) i	8	0.81	0.51	-
HB	NC	20	0.40 (08)	0.10 (02)	0.00 (00)	0.50 (10)	10	1.02	-	-
	BaP	20	1.05 (21) +	0.05 (01) i	0.20 (04) i	1.30 (26) +	26	2.66	1.64	-
	OC 0,25% + BaP	20	0.95 (19) i	0.05 (01) i	0.25 (05) i	1.25 (25) i	25	2.56	1.54	-
	OC 0,5% + BaP	20	0.65 (13) i	0.10 (02) i	0.10 (02) i	0.85 (17) i	17	1.74	0.72	-
	OC 1% +BaP	20	0,60 (12) i	0.00 (00) i	0.00 (00) i	0.60 (12) +	12	1.22	0.2	87%

^a Statistical diagnosis according to Frei & Würzler (1988): +, positive; -, negative; i, inconclusive. m, multiplication factor for the evaluation of significantly negative results. Significance levels a = b = 0.05; ^b Including rare *flr*³ simple spots; ^c Considering the *mwh* clones for single *mwh* spots and twin spots; ^d Small simple stains; ^e Large simple spots; ^f Twin spots; ^g Sum of all observed mutant spots; (NC): Negative Control, Chia oil (OC). Source: Authors.

OC at a concentration of 1% reduced damages by BaP in 87% (Table 3), probably because it neutralized the free radicals produced. According to Kelkel et al. (2010) turmeric and resveratrol are examples of anticarcinogenic compounds whose activity is attributed to antioxidant properties that inhibit free radicals from causing the peroxidation of cell membrane lipids or causing oxidative DNA damage, as both processes are important initiators of cancer development. Treatment with OC shows a significant reduction in the number of spots. Probably, the addition of OC offers protection against the harmful effects caused by mutagenic compounds such as DXR and BaP. Interestingly, increases in OC concentration have not resulted in a greater reduction of the spots induced by DXR, which leads us to conclude that even in small concentrations OC offers great protection against oxidative stress and other mechanisms that can lead to mutation and later the formation of cancer cells. The difference observed in the frequency of spots induced by BaP treatment in ST and HB lines is probably due to the greater metabolic capacity of the HB line, as accordingly to Smith et al. (2008), the BaP needs to be metabolized in the body to be considered a mutagenic compound.

Graf e Van Schaik, 1992 observed the greater sensitivity of the HB line to the detection of pro-mutagens and pro-carcinogens is due to the high levels of cytochrome-P450, Graf et al. (1983) which are xenobiotic metabolizing enzymes, present in the ORR line; *flr*³. Thus, the higher frequency of mutant spots observed in the HB strain is within the range described in the literature and does not represent deviations.

Our results reinforce that OC may have the antimutagenic capacity and the literature confirms the presence of antimutagenic substances. According to Ayerza e Coates, (2005) the main compound found in OC omega-3, which Calder (2012), describes as an agent with protective capacity against oxidative stress. Sargi et al. (2013) have also analyzed the antioxidant capacity and chemical composition of chia seeds and concluded it represents an excellent source of fatty acids with antioxidant activity and important compounds for human metabolisms, such as alpha-linolenic acid.

Marcinek e Krejpcio, (2017) explained that several species of the *Salvia* have protective properties against cancer and Mathew & Thoppil, (2012) found similar results to our work when analyzing extracts from *Salvia farinacea*, *Salvia microphylla* and *Salvia splendens* concluding the extracts of these three plants have antimutagenic effects.

4. Conclusion

The results indicate that the OC, at the evaluated concentrations of 0.25, 0.5 and 1%, it has no mutagenic effect and reinforces its protective activity when associated with the use of compounds with proven mutagenic capacity, DXR and BaP. In some treatments, OC reduced the spots number induced by DXR by up to 97%, suggested the use of oil as a preventive against oxidative stress.

Reference

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