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Avaliação do efeito genotóxico de *Ocimum basilicum* L. e Linalool

Assessment of genotoxic effect of *Ocimum basilicum* L. and Linalool

Evaluación del efecto genotóxico de *Ocimum basilicum* L. y Linalool

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Resumo

Ocimum basilicum pertence à família Lamiaceae, é uma planta conhecida popularmente como manjericão e pode ser encontrada em várias regiões do mundo, sendo o monoterpeno Linalol o constituinte majoritário do óleo essencial de *Ocimum basilicum* L. Medicinalmente, é considerado poderoso antisséptico, carminativo, digestivo, inseticida e analgésico, além de possuir atividades antibacterianas, antifúngica e antiviral comprovadas. Sabendo que avaliar a atividade genotóxica de plantas medicinais é extremamente importante como uma das etapas do estudo de toxicidade, este estudo teve como objetivo analisar o efeito genotóxico do óleo essencial de manjericão e de seu ingrediente ativo linalol. O teste de micronúcleos em células de sangue periférico foi usado neste trabalho. Grupos de três camundongos machos e três camundongos fêmeas receberam, via gavagem, os compostos na dose de 100 e 200 mg/kg do peso do animal. O grupo controle negativo recebeu apenas o dispersante da amostra (água destilada) e o controle positivo recebeu ciclofosfamida 50 mg/kg do peso do animal. 24 horas após o tratamento, os animais foram sacrificados, o sangue da veia caudal foi coletado e feito lâminas de esfregaço. Os resultados obtidos mostraram a ausência de efeito genotóxico dos compostos testados. Estudos futuros de toxicidade necessitam ser feito para que o uso desta planta no tratamento de doenças seja estimulado.

Palavras-chave: *Ocimum basilicum;* Micronúcleo; Linalol; Genotóxico.

Abstract

Ocimum basilicum belongs to the Lamiaceae family, is popularly known as basil and can be found in several regions of the world, being the monoterpene Linalol or the main component of the essential oil of *Ocimum basilicum L*. Medicinally it is considered powerful antiseptic, carminative, digestive, insecticide and analgesic, besides having proven antibacterial, antifungal and antiviral activities. Knowing that evaluating the genotoxic activity of medicinal plants is extremely important as one of the stages of the toxicity study, this study aimed to analyze the genotoxic effect of basil essential oil and its active ingredient linalool. The micronucleus test on peripheral blood cells was used in the study for activities. Groups of three mices males and three females received, by gavage, the compounds in dose of 100 and 200 mg/kg of animal weight. The negative control group received only the dispersant of the sample (distilled water) and positive control received Cyclophosphamide 50 mg/kg of animal weight. Twenty-four hours after treatment, the animals were sacrificed, blood was collected from the caudal vein and made a smear on the slide. The obtained results showed the absence

of genotoxic effect of tested compounds. Further studies of toxicity need to be made to the use of this plant in the treatment of diseases to be stimulated.

Keywords: *Ocimum basilicum*; Micronucleus; Linalool; Genotoxic.

Resumen

Ocimum basilicum pertenece a la familia Lamiaceae, es una planta conocida popularmente como albahaca y se puede encontrar en varias regiones del mundo, siendo el monoterpeno Linalol el componente principal del aceite esencial de *Ocimum basilicum* L. Medicinalmente, se considera un poderoso antiséptico, carminativo, digestivo, insecticida y analgésico, además de tener actividades antibacterianas, antifúngicas y antivirales comprobadas. Sabiendo que evaluar la actividad genotóxica de las plantas medicinales es extremadamente importante como una de las etapas del estudio de toxicidad, este estudio tuvo como objetivo analizar el efecto genotóxico del aceite esencial de albahaca y su ingrediente activo linalol.

La prueba de micronúcleos en células de sangre periférica se utilizó en este trabajo. Grupos de tres ratones machos y tres hembras recibieron, mediante sonda, los compuestos a una dosis de 100 y 200 mg / kg del peso del animal. El grupo de control negativo recibió solo el dispersante de muestra (agua destilada) y el control positivo recibió ciclofosfamida 50 mg / kg del peso del animal. 24 horas después del tratamiento, se sacrificaron los animales, se recogió sangre de la vena caudal y se hicieron portaobjetos. Los resultados obtenidos mostraron la ausencia de efecto genotóxico de los compuestos probados. Es necesario realizar futuros estudios de toxicidad para alentar el uso de esta planta en el tratamiento de enfermedades.

Palabras clave: *Ocimum basilicum*; Micronúcleo; Linalool; Genotóxico.

1. Introduction

It is known since antiquity population makes use of medicinal plants as an alternative source for the treatment of various diseases. So much in the form of extemporaneous preparations, as in the form of herbal medicines, the medicinal plants represent a valuable therapeutic source for both populations in developing countries development, with difficulties in accessing other therapeutic measures (Heinrich, 2010) and in developed countries, where populations seek complementary therapies and alternatives (Jütte *et al.*, 2017).

Medicinal plants and herbal medicines can be consciously chosen as an alternative to traditional medicine. According to data from the Ministry of Health, published in Portal Brazil, there was an increase in the search for treatments based on medicinal plants or herbal

medicines in the Unified Health System (SUS). In 2013, approximately 6 thousand people sought pharmacies or primary care seeking this type of treatment, in 2015 this number about 16 thousand people passed (Brazil, 2016).

Because they are natural, many adopted mistakenly the concept that they are not toxic to the body. However, this consumption should be monitored in order to warn of possible effects on living organisms, since they are often exposed to mutagenic substances that can cause cell damage (Costa & Menk, 2000).

Silva *et al*., (2015) reported that some teas and infusions of medicinal plants may contain toxic substances with mutagenic effects. There is a growing concern about the mutagenic and carcinogenic effect of genotoxic agents in exposed occupational populations or accidentally, or by lifestyle. Genetic toxicology tests are assays designed to detect direct or indirect genetic damage induced by chemical compounds. Fixation of DNA damage can result in gene mutations, loss of heterozygosity, chromosome loss or gain, and chromosome aberrations. These events may play an important role in many malignancies. Thus, identifying genotoxic/mutagenic effects is important for the risk/benefit assessment of substances, in particular those which are part of the dietary habits of any populations (Doppalapudi *et al*., 2007).

The micronucleus test "in vivo" is widely accepted by international agencies and government institutions as part of the recommended battery of tests to establish the evaluation and registration of new chemicals and pharmaceutical annually entering the world market and that may have mutagenic activity (Ribeiro *et al*., 2003).

Basil (*Ocimum basilicum* L.), a member of the Lamiaceae family is an annual herb which grows in several regions around the world. Traditionally, basil has been extensively utilized in food as a flavoring agent, and in perfumery and medical industries (Telci *et al.*, 2006). The leaves and flowering tops of the plant are perceived as carminative, galactogogue, stomachic and antispasmodic in folk medicine. However, recently the potential uses of *O. basilicum* essential oil, particularly as antimicrobial and antioxidant agents have also been investigated (Lee *et al*., 2005; Wannissorn *et al*., 2005).The studies in the literature suggest linalool, the monoterpen, as the main active agent responsible for antibacterial activity (Ravid *et al*., 1997).

Considering the absence of studies on the toxic effects of this plant, the aim of the present study was to evaluate the genotoxic activities of *O. basilicum* and linalool using the micronucleus test on peripheral blood cells.

2. Material and Methods

2.1 Kind of study

This is a research carried out with laboratory experiments (Pereira *et al*., 2018). carried out in the molecular biology laboratory and in the pharmaceutical technology laboratory at the Federal University of Paraíba (UFPB)

2.2 Compounds

The *Ocimum basilicum* L. essential oil and the monoterpene linalool were obtained commercially from Quinari, and Sigma Aldrich, respectively.

2.3 Chromatography essential oil *O. basilicum*

The oil was obtained from the leaves of plants, the extraction of components was made by steam distillation and the analysis method was gas chromatography high resolution. Chromatographic separation was performed using a DB-5 capillary column (30 m x 0.25 mm (HP). The temperature of the chromatographer oven was programmed from 50°C (3 min), 3°C/min, to 170°C. The temperature of injector and detector were 200 °C. The split 1/200 and detector FID 200ºC. The injection volume was 1.0 µL (0.5% concentrated in chloroform). Identification of individual components was based on their mass spectral fragmentation based on two computer library MS searches (Wiley 229) and retention index.

2.4 Animals treatment

The use of animals was approved by the Ethics Committee for Animal Research Laboratory of Pharmaceutical Technology / UFPB under registration number 0101/11. For the realization of experimental models were used five to six-week old albino Swiss mice (*Mus musculus*), weighing approximately 30 g from the Biotery Prof. Thomas George -UFPB. The animals were acclimated to the bioterium local conditions for about seven days before the experimental tests under temperature (21 ± 2 ° C) and controlled light-dark cycle of 12 hours. The animals were fed chow and water ad libitum and were distributed in the different experimental groups at random.

2.5 Micronucleus test

To perform the micronucleus test, the animals were sacrificed with xylazine (5 mg/kg) in accordance with existing regulations to prevent anxiety or fear (stress) (Andrade; Pinto; Oliveira, 2006) and then blood samples were collected from the caudal vein of mices.

The micronucleus test on peripheral blood cells was carried out as described by Hayashi *et al*. (1994), who concluded that bone marrow cells can be replaced by peripheral blood as material for the micronucleus assay. This is allowed because, alternatively in mice, the micronuclei can be analyzed in circulating normochromatic erythrocytes (NCE, erythrocytes), whereas the spleen of mice did not hijack the blood micronucleated erythrocytes.

Groups of three mices males and three females received, by gavage, the essential oil of *O.basilicum* or linalool in dose of 100 mg/kg to 200 mg/kg. The negative control group received only the dispersant of the sample (distilled water) and positive control received Cyclophosphamide 50 mg/kg of animal weight. Twenty-four hours after treatment, the animals were sacrificed, blood was collected from the caudal vein and made a smear on the slide.

2.6 Analysis of the slides

The slides were stained with Panotic and observed under an optical microscope (Zeiss) increasing 1000x (objective = 100 x with eyepiece = 10 x) for counting the micronucleus. Were assessed at least 2,000 NCE per slides (Hayashi *et al.*, 1994).

In this study, the presence of micronucleus in erythrocytes of mice in the positive control was not influenced by gender ($p>0,05$), so data were pooled to determine the average number of micronucleus to calculate the standard error of the mean and to assess differences between groups.

The data from the micronucleus assay were statistically analyzed using Student's t-test, comparing the treated groups with controls (Pereira, 1991). The significance level considered was $p<0.05$. Results were expressed as mean ± standard error of mean.

3. Results and Discussion

The detection of cytotoxic activity, genotoxic and / or mutagenic is a priority measure in the production of a herbal medicine , since various chemical compounds may be capable of causing toxic effects and even modify the genetic information contained in DNA. Obtaining data on the toxicity of these agents should be anticipated by experiments that can provide, with reasonable safety margin, an indication of the risks involved in their use (Benigni, 2005).

Through phytochemical prospecting of the essential oil of *O.basilicum*, it was possible to determine the presence of diverse compounds. The chromatography results are shown in Table 1. It was observed that the essential oil of *O.basilicum* presented as major compound linalool.

Table 1 - Chromatography of essential oil of *Ocimun basillicum.*

Compounds	%
α-pinene	0.4
β-pinene	1.1
Myrcene	0.7
1,8 cineol	8.8
trans-β -ocimene	0.6
Linalool	55.2
Terpinen-4-ol	0.9
Eugenol	3.2
β -Caryophyllene	0.4
Bergamotene	7.0
Germacrene D	2.2
γ- Cadineno	2.9
Muurolol	2.9

Source: Silva (2020).

The arial parts of *O.basilicum* are reported to have strong medicinal use like antimicrobial and antiviral property and with high vitamin and mineral content (Chiang *et al*, 2005). It contains a chemical, eugenol, which is antimicrobial.

Studies show that the chief constituents include chavicol methyl ether or estragole, linalool and eugenol (Hussain *et al*., 2008; Omidbaigi *et al*., 2003). The studies in the literature suggest the monoterpen linalool as the main active agent responsible for antibacterial activity.

The evaluation of micronucleus induction is the main test in vivo in a battery of genotoxicity tests and is recommended by enforcement agencies around the world as part of the safety assessment of chemicals and natural products. The Micronucleus Test (TM) has been used to evaluate the carcinogenic potential of compounds in toxicity studies (Balmus et al., 2015), in the genotoxicity screening of many classes of pharmaceutical chemicals, agricultural products, food additives, among others (Hayashi, 2016). In addition, TM is used to detect chromosomal damage caused by environmental and lifestyle factors, as well as occupational exposures and certain diseases (Nersesyan et al., 2014).

The results showed that the essential oil *O. basilicum* and its major compound linalool at doses of 100 and 200 mg/kg showed no genotoxic activity (Table 2), since the amount of micronuclei formed was significantly smaller than those formed in the groups treated with cyclophosphamide positive control (p<0,05).

Table 2 - Micronucleus frequency in 2000 found peripheral blood erythrocytes of mice of different experimental groups.

Experimental group	**Number of micronucleated erythrocytes (mean ± s.e.m)**
Negative control	1.5 ± 0.42***
Cyclophosphamide (50 mg/Kg)	43.5 ± 5,89
E.O *O. basilicum* (100 mg/Kg)	1.167 ± 0.3***
E.O *O. basilicum* (200 mg/Kg)	1.5 ± 0.34***
Linalool (100 mg/Kg)	3.33 ± 0.49***
Linalool (200mg/Kg)	5.3 ± 0.61***

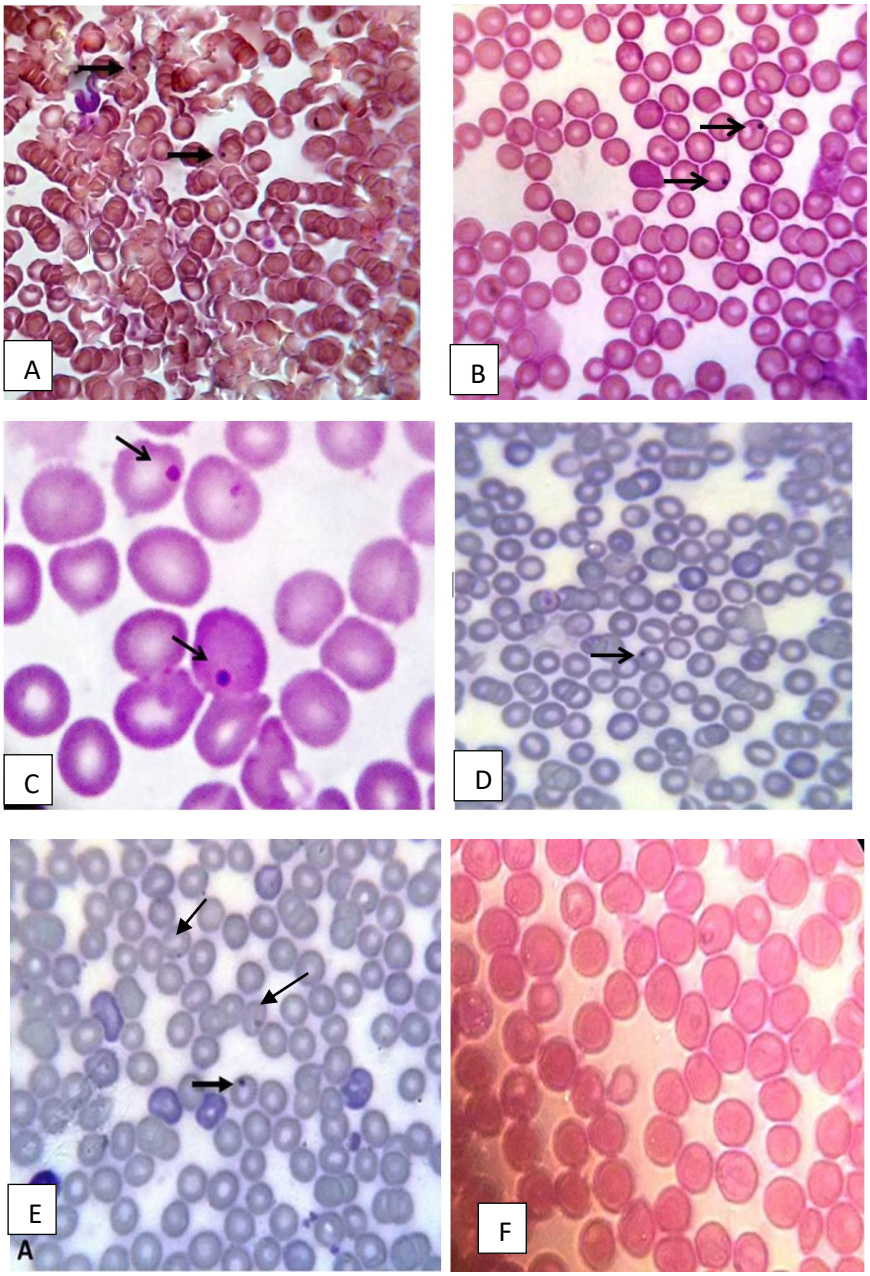
Tests were performed in triplicate (n = 6) with a confidence interval of 95%. The comparison between groups were performed using the *t* test for the program Graph Pad Prism 5. *** p <0.001 compared with positive control. Legend: E.O: Essential oil. Source, Silva, VA (2020).

Observing Table 2 above, it is concluded that the essential oil of basil and linalool in the tested doses, 100 mg / kg and 200 mg / kg of the body weight of the mouse, does not have a genotoxic effect since it did not induce the formation of micronuclei in the erythrocytes

significantly, with the amount of micronuclei found in the treated groups close to that found in the negative control.

The increase in the frequency of micronucleated or cross-linked micronucleated erythrocytes in rodents is indicative of the occurrence of chromosomal damage related to numbers or chemicals. In Figure 1 below, observe the presence of micronuclei found in the erythrocytes of the associated mice and in the control group.

Figure 1 - Micronucleus in mice red blood cells treated. **A**: *O.basilicum* at doses of 100 mg/Kg ; **B:** *O.basilicum* at doses of 200 mg/Kg; **C**: Linalool at doses of 100 mg/Kg; **D**: Linalool at doses of 200 mg/Kg; **E**: Ciclophosphamide 50 mg/kg; **F:** Negative control.



Fonte: Silva, VA (2020).

It is observed in Figure 1 above that in the control group (F), treated with water, no micronuclei are observed in the erythrocytes present in this field of view and when compared to the group treated with cyclophosphamide (Figure 1-E) already a higher frequency of micronuclei is observed, showing the carcinogenic effect of this compound. Few micronuclei are observed in the groups treated with the essential oil of basil and with linalool, corroborating the statistical data shown in Table 2.

Fletcher *et al*. (2005) evaluating the genotoxicity of *Malaleuca angustifolia* oil as well as the main oil component, 4- terpineol , and the results showed 4- terpineol showed toxicity only at the highest dose used.

Santos (2011) showed that the essential oil of oregano did not induce micronucleus formation in Wistar rats revealed no genotoxicity in the essential oil.

Through the results obtained, it can be concluded that the essential oil of *O.basilicum* e and your major compound linanool does not induce an increase in the frequency of the micronucleus characterized as an agent not mutagenic in these conditions. As noted in the discussion, the genotoxicity study is a widely used test in the scientific field to assess the mutagenic potential of substances, or what makes this work relevant.

4. Conclusion and Suggestions

Knowing that basil is a plant already used by the population for medicinal purposes, it is of great importance to know the toxicity of this plant, since it is not because they are natural that they cannot be toxic. In this study, the low genotoxicity of the essential oil of basil and linalool are promising results for the safe use of these compounds by the population, however other toxicity studies must be carried out to guarantee safety for their consumption for therapeutic purposes.

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