Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(3); 540-542

ISSN: 0975-4873

Research Article

Assessment of Genotoxic Effects of Flavonoid 5,7,4'-Trimethoxyflavone

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Available Online: 3rd May, 2015

ABSTRACT
The flavonoids are a large class of polyphenolic compounds found in plants that are known to exhibit biological effects. In the study, the flavonoid 5,7,4'-trimethoxyflavone was evaluated for its genotoxic effects. 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 flavonoid in dose of 300 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 flavonoid.

Keywords: Flavonoid, Genotoxic, Micronucleus, Polyphenols

INTRODUCTION

Flavonoids are a subgroup of the more extended family of polyphenols with a basic structure containing two benzene rings with a pyrane ring in the middle. Flavonoids are outstanding antioxidants and because of their antioxidant activity as well as their abundance in fruit and vegetables, they may partly contribute to the currently known health benefits of plant foods[1-3].

In drug development, the genotoxicity assays represent a considerable effort, as most pharmaceutical organizations evaluate a new therapeutic agent based on in vitro and in vivo data genotoxic[4]. In this context, tests to evaluate the genotoxic activity of the plants used by the population as well as their isolated compounds are necessary and important for establishing control measures in widespread use. Furthermore, it is necessary to clarify the mechanisms and conditions that mediate the pro- posed biological effect before plants are considered as therapeutic agents[5].

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 heterozygosis, 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[6].

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[7].

The in vivo rodent micronucleus assay has been accepted as a short-term assay for evaluation of the clastogenicity of chemical compounds as well as the anticlastogenicity of chemopreventive potential of compounds[8-12]. Mouse peripheral blood, instead of bone marrow cells, was introduced to use in the micronucleus assay[13].

Considering the absence of studies on the toxic effects of this flavonoid 5,7,4´-trimethoxyflavone, the aim of the present study was to evaluate the genotoxic activities for the compost using the micronucleus test on peripheral blood cells.

MATERIALS AND METHODS

Chemicals

The flavonoid 5,7,4'-trimethoxyflavone (TMF) was purchased from INDOFINE Chemical Company (Hillsborough, NJ). The Cyclophosphamide was purchased from Sigma Chemical Co (St. Louis,USA).

Animals treatment

The use of animals was approved by the ethics committee on animal use of the Biotechnology Center of the Federal University of Paraiba under registration number 0404/14. For the realization of experimental models were used five

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Table 1: Micronucleus frequency in 2000 found peripheral blood erythrocytes of mice of different experimental groups.

Experimental group	Number of micronucleated erythrocytes (mean±SEM)
Control (water)	12,7 ± 0,29
Cyclophosphamide (50 mg/kg)	43.5±5.89*
TMF (300 mg/kg)	12,6 ± 0,50

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 h. The animals were fed chow and water ad libitum and were distributed in the different experimental groups at random.

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)[14] 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)[15], 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 flavonoid in dose of 300 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.

Analysis of the slides

The slides were stained with Panotic and observed under an optical microscope (Zeiss) increasing 1000x (objective 100x with eyepiece 10x) for counting the micronucleus. Were assessed at least 2,000 NCE per slides[15].

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. The significance level considered was $p<0.05$. Results were expressed as mean ± SEM. For data analysis, we used the statistical program GraphPad Prism® version 5.0.

RESULTS AND DISCUSSION

The assessment of micronucleus induction is the primary *in vivo* test in a battery of genotoxicity tests and is recommended by law enforcement agencies worldwide as part of the safety assessment of chemicals and natural. The test, when performed correctly, detects both effects: clastogenic and aneugenic[16].

According Cammerer et al (2007)[17] the micronucleus assay done in peripheral blood has further advantages, such as the easy preparation of the sample small amount of blood, the speed in obtaining results, the ability to obtain repeated samples of the same animal, and the possibility of obtaining samples studies chronic.

Micronucleus in young erythrocytes emerge primarily from acentric fragments or chromosomes that are unable to migrate following the mitotic spindle during cell division of hematopoietic tissue[18,19]. An increase in frequency micronucleus test animals treated with different substances is an indication of chromosomal damage induced[16]. Micronuclei are indicative of numerical and/or structural chromosome aberrations during cell mitosis. Other authors have used the micronucleus test as a biomarker for chromosome instability and malignancy, observing higher frequencies of micronucleated cells among cancer patients than among healthy individuals[20,21].

The results showed that both at the dose of 300 mg/kg of the flavonoid did not induce a significant increase in the numbers of micronucleus in relation to the negative control ($p>0,05$) not presenting, therefore, clastogenic and aneugenic effect. Only cyclophosphamide (positive control) induced a significant increase in the amount of micronucleus ($p<0,05$) (Table 1).

CONCLUSION

The flavonoid TMF does not induce an increase in the frequency of the micronucleus characterized as an agent not mutagenic in these conditions. Further studies of toxicity need to be made to the use of this flavonoid in the treatment of diseases to be stimulated.

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