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STUDY OF ACUTE ORAL TOXICITY AND GENOTOXIC, MUTAGENIC, AND ANTIMUTAGENIC POTENTIAL OF THE METHANOLIC FRACTION OF *BUCHENAVIA TETRAPHYLLA* (AUBL.) R.A. HOWARD

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Abstract : *Buchenavia tetraphylla* is a tree reported as ethnomedical by communities in the semi-arid region, used as an expectorant, antitussive, against diarrhea, indigestion, and as a tonic against weakness. Studies with *B. tetraphylla* have shown antimicrobial activity promoted by its extracts and fractions; however, there is no information about its toxicity. The use of plant products should always be based on information about their chemical composition and safety of use, as they can be potentially toxic. In this sense, the objective of this study was to evaluate the acute oral toxicity, genotoxic and mutagenic potential *in vivo* and *in vitro*, and anti-mutagenic potential of the methanolic fraction of B. *tetraphylla (*BtMeOH). For the *in vivo* tests, the fraction was administered orally at a dose of 2000 mg/kg in Swiss albino mice. The animals were observed for 14 days after administration to assess acute oral toxicity. *In vivo* genotoxicity and mutagenicity were evaluated using the comet assay and micronucleus test, respectively, 48 hours after administration of the fraction. *In vitro* mutagenic potential was assessed using the Ames test. BtMeOH did not induce significant changes in acute toxicity analyses. The fraction did not induce an increase in the frequency or index of DNA damage. The number of micronuclei in the erythrocytes of animals treated with BtMeOH was similar to that of the negative control group. However, at a concentration of 100 mg/mL, the BtMeOH fraction showed a mutagenic index (MI) > 2.0 for strain TA97, indicating mutagenic action. At a concentration of 10 mg/mL, BtMeOH showed no evidence of mutagenicity for any of the strains tested. The fraction also managed to reverse mutations induced by mutagenic agents. These results indicate that BtMeOH was not significantly genotoxic or mutagenic, showing few changes in the DNA structures analyzed.

Keywords: Micronucleus; Comet assay; Ames test; Safety of use; DNA.

INTRODUCTION

The vegetation of the northeastern semi-arid region, especially the caatinga, comprises highly endangered plant formations and is still a little-known source of natural resources. This environment is home to representatives of the Combretaceae family, such as *Buchenavia tetraphylla*, which has a neotropical distribution and occurs naturally in Brazil in the North, Northeast, Central-West, and Southeast (Marquete; Loiola, 2015). This species is reported as ethnomedical by communities in the semi-arid region and indigenous groups (Agra; Freitas; Barbosa-Filho, 2007; Agra et al., 2008; Oliveira et al., 2012), being used as an expectorant, antitussive, against diarrhea, indigestion, and as a tonic against weakness (Agra et al., 2007). Few studies have been published on the genus Buchenavia, however, research with B. *tomentosa* and *B. tetraphylla* has shown antimicrobial activity promoted by extracts and fractions of this plant against strains, including resistant strains of gram--negative and gram-positive bacteria and fungi (Oliveira et al., 2012; Teodoro et al., 2015; Cavalcanti et al., 2017). In addition, organic extracts from the leaves of B. *capitate* have shown a cytoprotective effect against HIV in human lymphoblastoid cell culture (CEM-SS) (Beutler et al., 1992). However, there is insufficient scientific information about this plant.

The use of plant products must be based on information about their chemical composition and toxicity, as such products are not free from adverse effects and may be potentially nephrotoxic, hepatotoxic, mutagenic, or genotoxic (Oliveira et al., 2016). Several biomarkers are recognized by international regulatory agencies and used to assess the safety and efficacy of natural products (Bhagat, 2017). Among the most widely used tests are the Ames mutagenicity test, the micronucleus (MN) test, and the comet assay, which are capable of detecting significant damage to genetic material and specific mutations (Matsumoto et al., 2017).

Thus, the objective of this study was to evaluate the acute oral toxicity, genotoxic and mutagenic potential of the methanolic fraction of *B. tetraphylla* in polychromatic erythrocytes of mice. We also verified its mutagenicity in recombinant strains of *Salmonella typhimurium* in order to provide initial preclinical information on this species.

MATERIALS AND METHODS

BOTANICAL MATERIAL AND OBTAINING THE METHANOLIC FRACTION

Buchenavia tetraphylla leaves were collected in November 2013 at Catimbau National Park (Pernambuco, Brazil). They were dried in an air circulation oven and herbarium--preserved, with a specimen properly identified and deposited in the Herbarium of the Agronomic Institute of Pernambuco (IPA) as reference specimen number 84.104. After dehydration, the material was ground in a Macsalab mil knife mill (Model 200 LAB) and the powder was stored in dark jars until use. First, an extraction was performed with hexane (100 mL/100 g dry weight) under constant stirring at 125 rpm for a period of 72 hours until exhaustion. The material was filtered and the liquid part concentrated in a rotary evaporator, with the residue undergoing further extractions, following a polarity gradient, with the solvents chloroform, ethyl acetate, and methanol. After fractionation, the methanolic fraction (BtMeOH) was stored in a freezer at 4°C for later use.

ANIMALS

Male or female Swiss albino mice, approximately eight weeks old and weighing around 40 g, were used. During the experiment, the animals were kept in polypropylene boxes

maintained at a temperature of 22°C ± 2°C, relative humidity of 50 ± 5%, with a 12-hour light/dark cycle. The animals received drinking water and feed ad libitum. All experimental procedures complied with the standards suggested by the Brazilian College for Animal Experimentation (COBEA) and were approved by the Ethics Committee on Animal Use of the Center for Biological Sciences of the Federal University of Pernambuco (CEUA-UFPE) (protocol no. 00049/2016).

ACUTE ORAL TOXICITY ASSESSMENT

The methodology recommended by the Organisation for Economic Cooperation and Development (OECD, 2001) was used to assess the toxicity of the BtMeOH fraction. The method allows the material being tested to be classified into different toxicity classes defined by fixed LD50 (median lethal dose) cut-off values (Reddy, 2015). The acute toxicity test consisted of administering a dose of 2000 mg/kg of the product under study by gavage. Two groups were used, each containing three animals (females), totaling six animals for the experiment. This procedure was performed in duplicate. The MeOH fraction was solubilized in 0.9% (w/v) NaCl and 1% DMSO, and one group received only the vehicle. After administration, the animals were observed continuously for the first two hours and then every 24 hours daily for 14 days to check for any behavioral changes or changes in physiological activities. The animals were evaluated using the Hippocratic screening method, which checks for any impairment in the animal, such as: state of consciousness, disposition, activity and coordination of the motor system and muscle tone, activity of the central and autonomic nervous systems of the animal. Water and feed consumption and organ weight (liver and kidney) were also observed after the end of the experimental period. Blood was also collected from the animals to check the biochemical parameters of total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), and urea, using specific kits (Labtest Diagnóstica, Lagoa Santa, Brazil).

Histological analyses of the liver and kidney of animals in the control and fraction groups were performed using optical microscopy. Organ fragments were fixed in formalin (10% v/v), then dehydrated in a series of ethanol solutions (70-100%), diaphanized in xylene, and embedded in paraffin. Histological sections of 5 μm were cut in a microtome, stained with hematoxylin-eosin, and mounted with Entellan resin (Merk-German) cover slips. The slides were observed under an optical microscope coupled to a Moticam 1000 1.3 MP digital camera (Motic Incorporation Ltd, Causeway Bay, Hong Kong).

IN VIVO GENOTOXICITY ASSAY

Groups of female mice (n=5) were treated with 0.9% saline solution (negative control), cyclophosphamide (25 mg/kg intraperitoneally, positive control), or BtMeOH (2000 mg/kg by gavage). The animals were anesthetized with a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg) 48 hours after treatment, and their blood (1 mL) was collected by retro-orbital puncture for use in the genotoxicity and mutagenicity assay.

The genotoxic activity of BtMeOH was evaluated using the Comet Assay (CA). First, 15 μL of the collected blood was homogenized with 100 μL of low-melting-point agarose, and the mixture was deposited on slides previously prepared with a standard agarose cover . The slides were kept at 4 °C for 10 minutes. After cooling, the slides without coverslips were subjected to a lysis solution for a period of 48 hours (2.5 M NaCl, 100 mM EDTA, 10 mM TRIS, 1% Triton X-100, 10% DMSO, pH 10). After lysis, the slides were subjected to elec-

trophoresis in a horizontal tank containing an alkaline buffer solution (1M NaOH and 200 mM disodium EDTA, pH 13) for 20 minutes, with a current of ± 300 mA and a potential difference of 32V. The slides that served as positive controls were exposed for 10 minutes to a 200 mM hydrogen peroxide solution. After electrophoresis, the slides were neutralized for 15 minutes in 0.4 M Tris-HCl buffer, pH 7.5, and fixed for 5 minutes in absolute alcohol. For development, each slide was stained with 30μL of ethidium bromide solution (0.0002%, w/v). The evaluation was performed under a fluorescence microscope (Zeiss-Imager, M2). One hundred nucleoids per animal were analyzed, observing the relationship between the tail length and the comet head size. Each nucleoid analyzed was classified into one of five classes: 0 (no damage); 1 (little apparent damage); 2 (moderate damage); 3 (moderate damage with longer tail); and 4 (maximum damage). The values obtained for each individual can range from 0 (totally intact: 100 cells x 0) to 400 (maximum damage: 100 cells x 4); we call this value the damage index (DI) per animal. Thus, the DI was calculated as follows:

Total DI = 0 (number of class 0 comets) + 1 (number of class 1 comets) + 2 (number of class 2 comets) + 3 (number of class 3 comets) + 4 (number of class 4 comets)

Another parameter analyzed was the damage frequency (DF%), which was calculated according to the percentage of all nucleoids with some damage (class 1 to class 4) in relation to the total number of nucleoids counted, ranging from class 0 to class 4 (total number) (COLLINS et al., 2008), following the formula:

$$DF = \frac{[\ (\text{total number} - \text{class 0 number}).100\ ]}{\text{total number}}$$

EVALUATION OF IN VIVO MUTAGENIC POTENTIAL

The peripheral blood micronucleus (MN) test was used to verify the *in vivo* mutagenic potential of BtMeOH (Hayashi et al., 1998). Ten microliters of blood sample from each animal were placed on two slides previously prepared with acridine orange, and the presence of micronuclei was evaluated using a fluorescence microscope (OECD, 2014). For each sample, 2000 polychromatic erythrocytes were counted, and a blind analysis was performed by a single individual.

MUTAGENIC ACTIVITY BY THE SALMONELLA TYPHIMURIUM ASSAY **(AMES TEST) AND** *ANTIMUTAGENICITY*

The mutagenicity test was performed according to the parameters established in the OECD Guideline for Testing of Chemicals (2014). Analyses were performed with *Salmonella typhinurium* strains TA97, TA98, and TA102 with alterations in the histidine genes, making them histidine-dependent. In the presence of a mutagenic agent, colonies may undergo further mutations and reverse their histidine dependence. The strains used in this test included those with (+his9) and without (-his9) metabolism, with the aim of selecting the doses used in the tests and excluding concentrations that presented cytotoxicity. The concentrations of BtMeOH used were 10 and 100 mg/mL. Cytotoxicity was detected based on the reduction of revertant colonies and the absence or decrease of control colonies. The BtMeOH fractions were added to test tubes together with the bacterial culture solution of S. *thyphimurium* TA97, TA98, and TA102, phosphate buffer (pH 7.4), and 2 mL of agar supplemented with 0.5 mM histidine and biotin. The addition of the S9 metabolic activator was performed in another triplicate assay, where the BtMeOH fractions were distributed

and added together with the bacterial culture solution of S. *thyphimurium* TA97, TA98, and TA102 bacterial culture solution, 500 µL of the S9 metabolic activator solution with a final concentration of 8% of the total volume, and 2 mL of surface agar supplemented with 0.5 mM histidine and biotin. The solutions were homogenized and plated on Petri dishes containing Minimal Medium and incubated for 48 hours in a $_{CO_2}$ incubator at a temperature of 37°C. DMSO was used as a negative control, 4-Nitro-o-phenylenediamine (NPD) as a positive control for TA97-98, and Mitomycin C (MMC) as a positive control for TA102. The antimutagenicity test was performed for negative results in the Ames test, associated with the known mutagens already described. The number of revertant colonies per plate was counted using a colony counter after the incubation period.

STATISTICAL ANALYSIS

The acute toxicity results were submitted to one-way analysis of variance (ANOVA) followed by Newman-Keuls test with a 95% confidence interval when compared to the control group. The MN and EC results were analyzed by comparing the treated group that received BtMeOH with the negative control group using the Mann-Whitney test. To verify the efficiency of the tests, the negative (NC) and positive (PC) control groups were also compared using the Mann-Whitney test. The significance level established in all tests was $p \leq 0.05$, and R software was used for all analyses (RDEVELOPMENT, 2012). For the analysis of the AMES and antimutagenicity tests, the Fisher test was used in the R Studio program, with a significance level of $p \leq 0.05$.

RESULTS

EVALUATION OF ACUTE ORAL TOXICITY

The results of this test are shown in Table 1. The animals received a dose of 2000 mg/kg of BtMeOH orally. In the first hour of observation during the Hippocratic screening, immediately after gavage, the treated animals showed piloerection and drowsiness, but none died within 24 hours. There was no significant variation in weight or water and feed consumption compared to the control group animals during the 14 days of observation. No significant differences were observed in organ weight and macroscopic appearance. The results obtained indicate that BtMeOH had low toxicity with $_{an}$estimated LD$(_{50)}$of 2500 mg/kg.

Regarding the biochemical parameters evaluated, there was a statistical difference in the TGO (AST) enzyme values of the animals treated with BtMeOH compared to the control. No other biochemical parameter evaluated varied significantly (Table 2).

As for histopathological analyses, the results showed that the livers of the groups of animals treated with BtMeOH at a dose of 2000 mg/kg presented externally a thin capsule composed of fibrous connective tissue, Glisson's capsule, a well-developed parenchyma, and a vascular network with vessels of various calibers (Fig. 1A and 1C). The hepatocytes presented polyhedral morphology, central nucleus, prominent nucleolus, and acidophilic cytoplasm (Fig. 1B and 1D), with no morphological differences between the control group and the group after 14 days of treatment.

The results also showed that the kidneys of all experimental groups did not show morphological changes, presenting a well-developed cortical region (Fig. 2A) and a characteristic medullary region (Fig. 2B). In addition, the renal tubules (Fig. 2C) and glomeruli were preserved in all animals analyzed (Fig. 2D).

Groups	Feed intake (g)	Water consumption (mL)	Initial/final animal weight (g)	Organ index	
				Liver	Kidney
BtMeOH	13.7 ± 2.0	15.7 ± 4.9	37.8 – 38.6	1,596	0.344
Control	15.5 ± 3.8	19.6 ± 4.7	37.4 – 36.3	1,696	0.367

Results expressed as mean ± standard deviation. *p < 0.05. Significant after one-way analysis of variance (ANOVA) followed by Newman-Keuls test with 95% confidence interval, when compared to the control group.

Table1 . Evolution of body weight, feed and water consumption, and organ weight (mean ± standard deviation) of animals treated with the methanolic fraction of *B. tetraphyla* in relation to the control group .

Treatments	Parameters					
	AST (U/L)	ALT (U/L)	FA (U/L)	ALB (g/dL)	PT (mg/dL)	UREA (mg/dL)
Control	77.05 ± 4.04	58.79 ± 8.63	30.47 ± 19.38	2.76 ± 0.39	4.36 ± 0.28	0.41 ± 0.06
BtMEOH (2000 mg/kg)	120.90 ± 21.99*	127.74 ± 38.33	37.41 ± 16.17	2.59 ± 0.40	4.34 ± 0.2	0.60 ± 0.04

Results expressed as mean ± standard deviation. *p < 0.05. Significant after one-way analysis of variance (ANOVA) followed by Newman-Keuls test with 95% confidence interval, when compared to the control group.

Table2 . Biochemical parameters in the blood of animals treated orally with BtMeOH for 14 days.

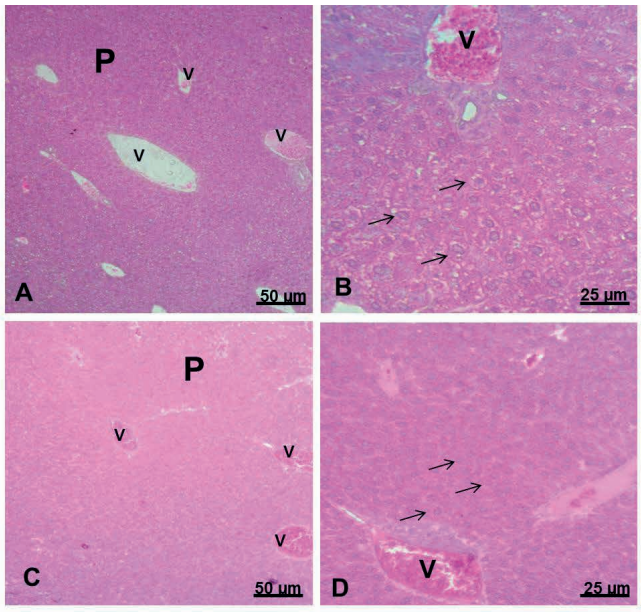

Figure1 : Histological analysis of liver toxicity 14 days after treatment with BtMeOH 2000mg/kg**. A**- Liver of the control group showing a well-developed parenchyma (P) and a vascular network with vessels of various calibers (V). **B**- Hepatocytes of the control group with polyhedral morphology, central nucleus, prominent nucleolus, and acidophilic cytoplasm (arrow), in addition to the presence of vessels. **C**- Liver of the treated group showing a parenchyma (P) and vessels of various calibers (V). **D**- Hepatocytes of the treated group with polyhedral morphology, central nucleus, prominent nucleolus, and acidophilic cytoplasm (arrow), in addition to the presence of vessels.

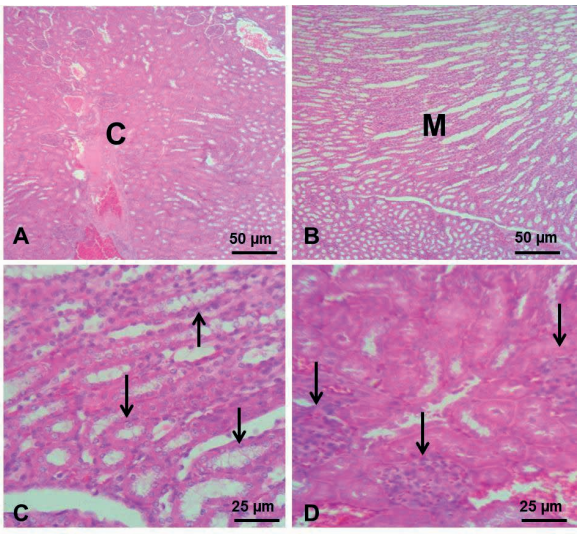

Figure2 : Histological analysis of kidney toxicity 14 days after treatment**. A**- Kidneys from the control group showing a well-developed cortical region (C). **B**- Kidneys from the control group showing a characteristic medullary region (M). **C**- Kidneys from the treated group with well-preserved renal tubules (arrows). **D**- Glomeruli from the treated group similar to the control group (arrows).

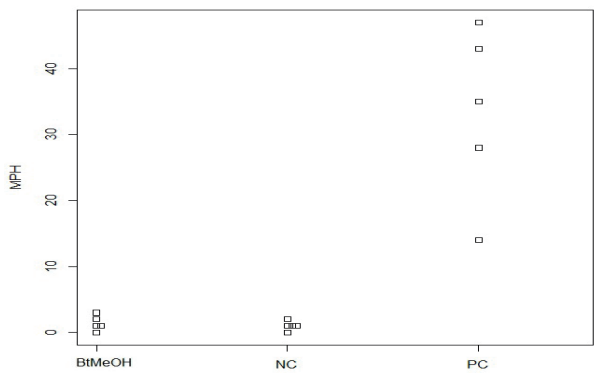

Figure3 **:** Evaluation of the *in vivo* mutagenicity of BtMeOH (2000 mg/kg b.w. v.o.) through the frequency of micronuclei in murine polychromatic erythrocytes (MPH). Each point represents one animal (n=5) in the group. NC: negative control. PC: positive control (cyclophosphamide 25 mg/kg b.w. i.p.).

Tests	CN	BtMeOH	CP
ID	53.4 ± 3.353	62.4 ± 2.274	351 ± 3.131*
FD	28.4 ± 1.520	42.4 ± 1.252	100 ± 0

Comet assay in peripheral blood of mice treated with BtMeOH (2000 mg/kg) through analysis of the Damage Index (DI) and Damage Frequency (DF). NC: negative control. PC: positive control (cyclophosphamide 25 mg/kg b.w. i.p.). Results expressed as Mean ± Standard Deviation. * $P < 0.05$ = significant difference from the negative control.

Table3 : Comet Assay (CA) test results.

EVALUATION OF GENOTOXICITY AND MUTAGENESIS IN VIVO

As there was no high toxicity at the dose of 2000 mg/kg, we used this dose for the genotoxicity and mutagenicity tests. Figure 1 shows the number of micronuclei in polychromatic erythrocytes of animals from the control and treated groups, sacrificed after 48 hours with the specific treatments. It can be observed that the number of polychromatic erythrocytes in BtMeOH was similar to the negative control (>10), with no statistically significant difference between them ($p > 0.05$). Thus, the BtMeOH fraction was not mutagenic. However, the presence of micronuclei in animals treated with cyclophosphamide (positive control) was significant.

In vivo genotoxicity was also analyzed 48 hours after BtMeOH administration, where DNA damage frequency and index were verified. The results showed that there was no statistically significant increase in FD or ID in the samples from animals treated with the fraction, compared to the negative control (Table 4). The group treated with cyclophosphamide showed an increase in DNA damage, as expected.

AMES TEST AND ANTIMUTAGENICITY

The data from the mutagenicity tests with the mutant strains *S. Thyphimurium* TA97, 98, and 102 are presented in Table 5. The results showed that at a concentration of 100 mg/mL, the BtMeOH fraction had a mutagenicity index (MI) > 2.0 for TA97, indicating that the fraction had significant mutagenic action: BtMeOH ($_{100\ mg/mL}$ (-his9) 34.2 ± 5.1 (2.0) and (+ his9) 30.0 ± 2.1 (1.4). At a concentration of 10 mg/mL BtMeOH, there was no mutagenic evidence for any of the strains tested and, therefore, it was evaluated for the antimutagenic test using the strains S. *thyphimurium* TA98 and TA102. The BtMeOH($_{10)\ (0)\ (mg/mL)}$+ NPD fraction showed >40% antimutagenicity with 462±22 revertant strains (42%) for TA98 (-his9), and BtMeOH($_{10mg/mL)\ +}$MMC showed low antimutagenicity with 1284±20 revertants (15%) for TA102 (-his9).

DISCUSSION AND CONCLUSION

Plants have been used for therapeutic purposes for many years, and their compounds are used as models for the synthesis of many drugs in the Western world (Kim et al., 2017; Rhalp; Helm, 1993). Chemical studies on the genus Buchenavia have pointed to the presence of flavonoids such as kaempferol and epicatechin, protoanthocyanidins, leucoanthocyanidins, quinic acid, hydrolyzable tannins such as gallic acid, and triterpenes (Oliveira et al., 2012; Teodoro et al., 2015; Cavalcanti et al., 2017). In phytochemical tests of the methanolic fraction of B. *tetraphylla* previously conducted by our group, we found polyphenols from the flavonoid group (quercetin) and hydrolyzable tannins (Silva et al., 2017). These metabolites are associated with the plant's biological activities as an antimicrobial against gram-negative and gram-positive bacteria and fungi of pathogenic interest (Oliveira et al., 2012; Cavalcanti et al., 2017).

Despite the recent discovery of biological properties and the ethnomedicinal use of this species, there are no studies on its oral toxicity or that of other species of the genus Buchenavia, making this a pioneering study. However, studies conducted with the Combretaceae family show that some extracts or compounds from representatives of the group have low toxicity. In an analysis of the acute oral toxicity of the species *Conocarpus erectus,* all animals subjected to a dose of 2000 mg/kg survived (Nascimento et al., 2016).

Jayesh et al. (2017) investigated the acute oral toxicity of *Terminalia bellirica* and found that at the maximum dose of 2000 mg/kg there were no significant changes in water consumption, feed intake, body weight, or histo-

logical changes or changes in liver and kidney function compared to the control group.

In our study, no histological changes were found in the kidney or liver of the group treated with BtMeOH at a dose of 2000 mg/kg. The renal tissues showed no signs of degeneration of Bowman's capsule, glomeruli, or proximal or distal convoluted tubules. No evidence of hepatotoxicity, such as vacuoles, lesions, or necrosis, was found. Female mice were used in the acute oral toxicity tests, as they are often used to determine the LD50 due to their greater sensitivity (Jayesh, 2017). The LD50 found in this study for the BtMeOH fraction according to the guidelines established by OECD 423 (OECD, 2001) was estimated to be above 2000 mg/kg. The aforementioned protocol does not recommend toxicity testing for doses of 5000 mg/kg due to the difficulty of solubilization and administration, except in cases where a study is necessary to ensure human health quality.

The micronucleus test is considered a reference for evaluating changes in chromosome structures (Oliveira et al., 2016; Haase et al., 2017). It indicates whether the substance is mutagenic based on the increase in the number of micronuclei in peripheral blood erythrocytes. The results show that BtMeOH at a dose of 2000 mg/kg administered orally was not mutagenic, with no statistical difference in the number of micronuclei compared to the negative control. Mendonça et al. (2016) found similar results for the aqueous extract of *Cecropia pachystachya* administered orally at a dose of 500-2000 mg/kg.

On the other hand, the comet assay is highly sensitive because it allows the identification of genotoxic agents that induce single--strand breaks in DNA or alkali-labile sites by measuring fragments (Mendonça et al., 2016; Traesel et al., 2017). The methanolic fraction of Buchenavia was not genotoxic *in vivo,* showing no significant damage to DNA structure when administered orally. Traesel et al. (2017) evaluated the mutagenic potential of Pequi oil (*Caryocar brasiliense)* through the in *vivo* comet assay, finding no significant DNA damage at any of the doses tested (125, 250, 500, 1000 mg/kg) compared to the negative control. In contrast, analyses performed with the monoterpene isolate β-phellandrene at doses of 1425 and 2859 mg/kg induced significant breaks in the DNA chain of splenic cells after administration in mice (Cheng et al., 2017).

In vitro mutagenicity in *S. thyphimurium* strains showed that BtMeOH was cytotoxic at a concentration of 100 mg/mL for strain TA97 (IM>2), while at 10 mg/mL the product was non-toxic for all strains tested. A study conducted with the hydroalcoholic extract of *Terminalia captata,* belonging to the Combretaceae family, showed that at low concentrations (13.12 mg/mL) the product induced an increase in revertant strains without metabolization TA 97 and 100, with a mutagenicity index greater than 2.0 (Mininel et al., 2014). *The* aqueous extract of *Cecropia pachystachya* increased the number of TA97 revertant strains without metabolic activation; however, it was not mutagenic for strains with metabolism (Mendonça et al., 2016).

The AMES test is used to assess whether the substance is capable of inducing specific genetic mutations, as each strain is mutated differently in the histidine operon. The TA97 and 98 strains are capable of detecting mutagens that shift the DNA reading frame to –C-C-C-C-C-C +1 cytosine and to –C-G-C--G-C-G-C-G-, respectively. Meanwhile, the TA102 strain detects agents that induce bridges between DNA strands and mutations by base pair substitution (Zhanataev et al., 2017). Yun et al. (2016) studied the mutagenic potential of *Artemisia capillaris* through the Ames test using strains TA98, TA100, and TA102, and observed that the aqueous extract was not

Treatment (mg/mL)	Mutagenicity						Antimutagenicity			
Bt-MeOH (mg/mL)	TA 97		TA 98		TA 102		TA98		TA102	
	- his9	+ his9	- his9	+his9	- his9	+ his9	- his9	+his9	- his9	+ his9
10	106 ± 22 (1.3)	119 ± 19 (1.2)	26 ± 1.5 (1.1)	34 ± 1.2 (1.4)	398 ± 30 (1.0)	390 ± 46 (1.0)	462±22	103±0.1	1284±20	98±54.32
100	34.2±5.1 (2.0)*	30±2.1 (1.4)	26 ± 0.3 (1.0)	24 ± 1.5 (1.0)	384.2±51 (1.1)	390±21 (1.0)	-	-	-	-
CP	747±6.4	821±9.2	789±5.1	902±7.4	970±4.9	1019 ± 4.9	-	-	-	-
CN	108±6.4	140±9.1	43±8	20±4	170±6.4	196±9.2	-	-	-	-

NC: Negative control (DMSO 100 µL/plate). PC: Positive control (NPD for TA97-98 and MMC as positive control for TA102. CONCENTRATIONS). *p<0.05: significant difference compared to control.

Table4 . Mutagenic activity expressed by the mean number of revertants/plate± , standard deviation, and mutagenicity index (MI) induced by BtMeOH in the presence (+S9) and absence (-S9) of metabolic activation, with *Salmonella typhimurium* strains TA97, TA98, and TA102**.**

mutagenic in any of the strains with and without metabolism, at concentrations ranging from 3.125 to 5 mg/mL.

When incubated with specific mutagenic agents, BtMeOH showed an antimutagenicity percentage of 15% (10mg/mL) for TA102 and 40% (100 mg/mL) for TA98, both without metabolism. A study conducted with *Camellia sinensis* showed that at concentrations greater than 1 mg/mL, its aqueous extract exhibited 40% antimutagenicity in relation to the TA98 strain without metabolism (Charehsaz et al., 2017).

Based on the results obtained, we can conclude that the methanolic fraction of *B. tetraphylla* did not cause significant changes in toxicity when administered orally, and was not capable of inducing genetic damage or mutations in any of the tests performed *in vivo*. *B. tetraphylla* is an important source of metabolites whose bioactivity is frequently reported in the literature. The results obtained in this study contribute to determining the safety of the medicinal use of B. *tetraphylla* leaves by the population.

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