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Toxicogenetic profile of rats treated with aqueous extract from *Morinda citrifolia* fruits

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Morinda citrifolia **((Family: Rubiaceae)) is extensively used in traditional medicine due to its anti-inflammatory, antimicrobial, antitumoral, and anti-hypertensive activities. However, there is no substantial data about hepatotoxic and toxicogenetic effects. This study evaluated biochemical changes and hepatotoxic, genotoxic, and mutagenic effects of aqueous extract of the fruit of *M. citrifolia* (AEMC) in liver, bone marrow, and peripheral blood cells. Animals (*Rattus novergicus*, 5 males and 5 females) were divided into negative control, positive control (Cyclophosphamide 25 mg/kg), and AEMC (2.5, 5, and 10 mg/kg, by gavage). AEMC induced increase of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), especially at 10 mg/kg in female (174.8 ± 50.7, 221.4 ± 24.6, and 174.7 ± 14.3 U/L) and male (156.5 ± 21.6, 183.7 ± 21.5, and 147.3 ± 17.8 U/L) (p<0.05). Histological analysis of livers showed inflammatory cell infiltration, nuclear fragmentation, microvacuolization, cellular swelling, points of inflammatoy necrosis, and discrete microvesicular steatosis. DNA damage in hepatocytes was found in both genders, mainly at 10 mg/kg (Frequency of Damage: 78.1 ± 4.5 and 70.4 ± 7.3%; Index of Damage: 107.6 ± 14.2 and 136.0 ± 26.9 for male and female, respectively). Similar results were observed in bone marrow cells. The AEMC 5 and 10 mg/kg induced micronucleus formation (4.4 ± 0.8 and 7.8 ± 1.1; 7.4 ± 1.1 and 9.6 ± 1.4 for peripheral blood and bone marrow cells, respectively) (p<0.05). These findings suggest clastogenic and/or aneugenic effects and genetic instability activated by AEMC, indicating precaution regarding the consumption of formulations or folk preparations based on this plant.**

Key words: Hepatotoxicity, genotoxicity, mutagenicity, *Morinda citrifolia*, noni.

INTRODUCTION

Popular knowledges associated with geographic and economic barriers to obtain health care contribute to the use of medicinal plants such as teas and extracts, especially in developing countries to supply primary basic

requirements (Firenzuoli and Gori, 2007; WHO, 2011; Akram et al., 2014; Tuttolomondo et al., 2014; Araujo et al., 2015). However, continuous exposure to synthetic or natural chemicals present in these preparations may lead to structural and functional damage to macromolecules, due to possible toxic effects, which can be measured by several genotoxic and mutagenic testing methodologies (Speit and Rothfuss, 2012; Hussin et al., 2014).

Morinda citrifolia Linn (Fam.: Rubiaceae) is habituated to the Southeast Asia and is distributed through several settlers of the Pacific Islands (Wang et al., 2002; Samoylenko et al., 2006; Ebeling et al., 2014). Aerial parts of this plant are commonly used in folk medicine as antibacterial, antiviral, antifungal, antitumoral, anthelmintic, contra-ceptive, hypotensive, anti-inflammatory, antioxidant, immunomodulating (Nayak and Shettigar, 2010), as well as antidopaminergic and antiadrenergic (Pandy et al., 2014). Moreover, its fruit has medicinal effects on allergy, arthritis, asthma, bacterial infections, cancer, diabetes, hypertension, menstrual disorders, obesity, gastric ulcers, headaches, sexual inhibition, insomnia, depression, stress, respiratory problems, AIDS, multiple sclerosis and drug dependency (Selvam et al., 2009; Gupta and Patel, 2013; Murata et al., 2014).

About 96 volatile compounds were identified in ripe fruit of *M. citrifolia* and more than 200 compounds were isolated from several parts of the plant, of which as hexanoic acid, octanoic and asperuloside acid, alcohols, esters, ketones and lactones are few of them. Nonetheless, the phytochemical composition is not complete yet (Potterat and Hamburger, 2007; Assi et al., 2015). Although folk uses of *M. citrifolia* fruits are an earlier report, still there is a lack of consistent data regarding hepatotoxic, genotoxic, mutagenic, and cytotoxic effects. Thus, studies addressing DNA damage in eukaryotic cells are important to undersand the risk of cellular injury. This study evaluated the possible biochemical changes and hepatotoxic, genotoxic and mutagenic effects of aqueous extract of the fruit of *M. citrifolia* (AEMC) in liver, bone marrow and peripheral blood cells in Wistar rats.

MATERIALS AND METHODS

Plant and extract preparation

Fruits of *M. citrifolia* were collected in 2014 in the municipality of Altos, Piauí, Brazil, (05° 02'20" S– latitude, 42° 27'39" O– longitude) at 187 m above sea level. The botanical identification was held at the Center for Environmental Sciences of Tropic Ecotonal Northeast, Teresina, Piauí (voucher number: 21644). After collection, the fruits were dried in a forced air oven for 8 days at a maximum temperature of 45°C (±1°C). Then, they followed by course grinding and were preserved in an amber glass.

HPLC analysis

High performance liquid chromatograph (HPLC) analysis was performed in AEMC to determine the presence of flavonoids and phenolic compounds. Briefly, Waters 2695 liquid chromatograph equipped with autosampler and a variable wavelength UV/VIS detector (Waters 2487 Detector Dual Absorbance, 190 to 700 nm). Columns: Waters Spherisorb ODS2 (5 µm, 4.6×250 mm). The mobile phases consisted of acetonitrile (A) HPLC grade purchased and 0.1% H_3PO_4 aqueous solution (B, filtered using a Millipore system). The following were the gradient conditions: 5% (A); 15% (A) for 10 min; 35% (A) for 40 min; 100% (A) for 15 min and 5% (A) for 5 min. Total run time was 70 min at a flow rate of 1.8 ml/min. Injection volumes were 20 µl. The AEMC was solubilized in acetonitrile (30%, HPLC grade and 0.1% H_3PO_4 aqueous solution, 70%).

Animals and treatment

A total of 50 Wistar adult *Rattus norvegicus* of both sexes weighing 200 to 250 g were purchased from the Faculty of Health, Life Sciences and Technology of Piauí (NOVAFAPI), Teresina, Piauí, Brazil. The animals were kept in plastic cages (6 animals/cage) with maintenance of diet (Purina, Brazil) and water *ad libitum* in air-conditioned temperature of 25°C with a photoperiod of 12 h light/dark cycle. The investigational protocols were approved by the local Ethical Committee on Animal Research at NOVAFAPI (Process No. #0039/10) and are in accordance with the national (*Colégio Brasileiro de Experimentação Animal* – COBEA) and international standard for the care and use of experimental laboratory animals (EEC Directive, 1986).

Animals (10 per group: 5 males and 5 females) were randomly distributed into the following groups: negative control (NC; untreated animals); positive control (Cyclophosphamide 25 mg/kg, i.p.) and three for AEMC (2.5, 5 and 10 mg/kg, by gavage). Animals of the positive control received cyclophosphamide (CPA) for 2 days and were sacrificed on the 3rd day. After 14 days of treatment, the rats were intraperitoneally anaesthetized with a solution composed of ketamine and xylazine at a dose of 40 and 5 mg/kg, respectively. Then, blood (5 ml) from the portal vein was collected to evaluate biochemical parameters. For the micronucleus test, blood was collected from the caudal vein. Liver tissue samples were collected for histopathological analysis and comet assay. In addition, bone marrow samples from both femurs were collected for micronucleus test and comet assay.

Biochemical parameters

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) were measured by using commercially available assay kits (Labtest Diagnóstica S.A., Brazil).

Liver histopathological analysis

For histological analysis, the livers of the remaining animals of each group were fixed in 10% formalin for 72 h. To examine

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morphological changes by light microscopy (Olympus, Tokyo, Japan) at a magnification of 200 and 400X, small pieces were processed, embedded in paraffin and 3 to 5 µm thick sections were prepared and stained with Hematoxylin and Eosin (H&E).

Comet assay

The alkaline comet assay was performed by the method earlier described by Speit and Rothfuss (2012). Briefly, 10 µl of the cell suspension (hepatocytes and bone marrow mononuclear cells) was mixed with a thin agarose layer low melting point (0.75%) (90 µl) and placed on pre-covered slides with normal melting point agarose (1.5%). The slides were immersed in lysing solution (2.5 mol/L NaCl, 100 mmol/L EDTA and 10 mmol/L Tris, pH 10 with 1% Triton X-100 and 10% of DMSO) up to 72 h at 4°C. The slides were then incubated in alkaline buffer (300 mmol/L NaOH and 1 mmol/L EDTA; pH >13) for 20 min followed by exposition to an electrical current 300 mA and 25 V (0.90 V/cm) for 15 min (electrophoresis). Finally, the slides were neutralized with Tris buffer 0.4 M, pH 7.5 and stained with silver solution. The results were expressed in index of damage (ID) and frequency of damage (FD). The ID was calculated from the visual assessment of the damage classes (0 to 4), extracting an index that expresses the overall damage suffered by the cells (100 cells/slide in duplicate). However, in injured cells, the DNA migrates from the core to the anode during electrophoresis, showing a similar comet tail. From these images, the cells were sorted between classes 1 (minimal damage) and 4 (maximum damage), 0 denoted for intact nucleus. The FD was calculated based on the equation: $FD = 100 - N_0$, where FD is the frequency of damage; N_0: number of tailless cells.

Micronucleus frequency assay

The micronucleus test was carried out according to Mavournin et al. (1990) and Kasamoto et al. (2013). Briefly, the collected bone marrow was mixed with 0.3 ml of fetal bovine serum on the slide previously coded. Additionally, peripheral blood was collected from the tails of live animals. The smears were performed by extender blade and after 30 min of drying, the slides were fixed in methanol for 10 min. Then, the slides were stained with Giemsa (Merck) in phosphate buffer 0.2 mol/L, pH 5.8. The counting of normochromatic erythrocytes (NCE), polychromatic erythrocytes (PCE) and micronuclei in polychromatic erythrocytes (MNPCE) were carried out by photomicrography at 100X. In each animal, 2000 PCE were analyzed. The ratio of PCE/NCE was determined by evaluating the frequency of PCE in 500 erythrocytes per animal.

Statistical analysis

Results were expressed as mean ± standard deviation (SD) (n=10). In order to determine the differences, data were compared by one-way analysis of variance (ANOVA) followed by the Tukey test (p<0.05).

RESULTS

Identification of compounds by HPLC analysis

Qualitative HPLC analysis of AEMC is as shown in Figure 1a. The correlation of chromatographic peaks was achieved by comparing experimental retention times (t_R) with reference standards (Figure 1b). All chromatographic operations were carried out in triplicate at ambient temperature. According to the chromatogram obtained for AEMC, gallic acid, chlorogenic acid, caffeic acid, ellagic acid, rosmarinic acid and flavonoid like-rutin with the following retention time (t_R) were found: 3,4,5-trihydroxybenzoic acid (1) t_R: 5.0 min; (1S,3R,4R,5R)-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,4,5 tri hydroxycyclohexane-1-carboxylic acid (2) t_R: 14.9 min; (E)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid (3) t_R: 15.1 min; 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R, 4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R, 4R,5R, 6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl] oxychromen-4-one (4) t_R: 32.5 min; 2,3,7,8-Tetrahydroxy-chromeno[5,4,3-cde] chromene-5,10-dione (5) t_R: 35.0 min; (2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxypropanoic acid (6) t_R: 37.5 min.

In vivo hepatotoxicity

Hepatotoxicity of AEMC in rats was observed by an increase (p<0.05) of the values of AST, ALT and ALP, especially at the highest dose (10 mg/kg) in female (174.8 ± 50.7, 221.4 ± 24.6 and 174.7 ± 14.3 U/L) and male (156.5 ± 21.6, 183.7 ± 21.5 and 147.3 ± 17.8 U/L) when compared with the negative control [(female: 32.1 ± 13.6, 41.1 ± 9.8 and 53.5 ± 2.7 U/L) and male (33.2 ± 14.8, 49.9 ± 12.0 and 59.7 ± 4.7 U/L), respectively]. Furthermore, high levels of ALP were found in all AEMC-treated animals (Table 1) and male animals also showed GGT increasing at the dose of 10 mg/kg (1.2 ± 0.2 U/L) in comparison with the untreated group (0.9 ± 0.1 U/L) (p<0.05). Similarly, histological analysis of livers also revelaed signs of injury, corroborating the alteration in serum enzymes, as morphological changes in both genders at 10 mg/kg, which showed histoarchitecture preservation with inflammatory infiltrating cells in the perivascular space (Figure 2a), nuclear fragmentation of hepatocytes (suggestive of apoptosis) (Figure 2b), microvacuolization (Figure 2c), cellular swelling (Figure 2d), points of inflammatoy necrosis (Figure 2e) and discrete microvesicular steatosis (Figure 2f), all findings indicative of hepatotoxicity.

DNA damage in liver and bone marrow cells

AEMC caused significant DNA damage (p<0.05) in hepatocytes of rats in all doses studied as described in Figure 3 (ID) and in Table 2 (FD). Once again, higher doses were more toxic for both genders, mainly at 10 mg/kg (FD: 78.1 ± 4.5 and 70.4 ± 7.3%; ID: 107.6 ± 14.2 and 136.0 ± 26.9 for male and female, respectively) when compared with NC (FD: 19.1 ± 6.9 and 20.6 ± 9.5%; ID: 36.8 ± 12.2). Similar results were observed in bone marrow, though, only higher doses (5 and 10 mg/kg) have demonstrated genotoxic activity (Figure 3 [ID] and Table 2 [FD]). There was increase with the frequency of

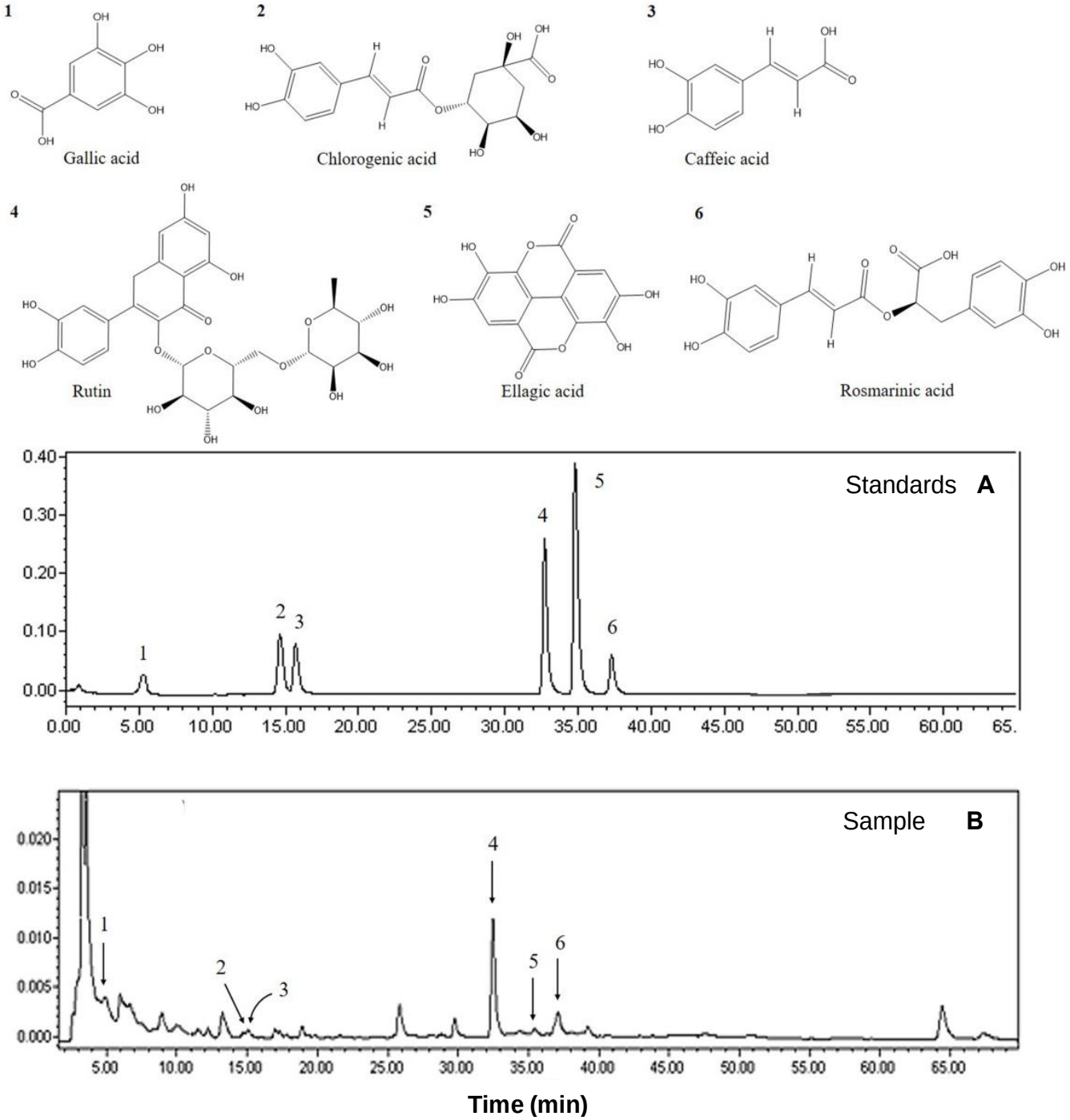


Figure 1. HPLC chromatogram of a standard mixture (A) and aqueous extract of the fruit of *M. citrifolia* (AEMC) (A). 3,4,5-trihydroxybenzoic acid (**1**) t_R: 5.0 min; (1S,3R,4R,5R)-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,4,5-trihydroxycyclohexane-1-carboxylic acid (**2**) t_R: 14.9 min; (E)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid (**3**) t_R: 15.1 min; 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one (**4**) t_R: 32.5 min; 2,3,7,8-Tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione (**5**) t_R: 35.0 min; (2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxypropanoic acid (**6**) t_R: 37.5 min.

damage at 5 mg/kg in bone marrow cells of males only (44.6 ± 4.4) and at 10 mg/kg it occurred in both genders (78.4 ± 5.7 and 68.1 ± 8.6, for male and female, respectively) (p<0.05). On the other hand, CPA, positive control, caused increased in FD and ID levels in an independent way of gender.

Table 1. Biochemical markers evaluated in rats treated with aqueous extract of fruit of *Morinda citrifolia* (AEMC).

Marker	Treatment									
	Negative control		CPA 25 mg/kg		AEMC					
					2.5 mg/kg		5 mg/kg		10 mg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
ALT (U/L)	32.1 ± 13.6	33.2 ± 14.8	263 ± 83.4*	267.1 ± 53.2*	80.8 ± 11.1	81.1 ± 28.2	129 ± 22.3*	107.1 ± 15.4*	156.5 ± 21.6*	174.8 ± 50.7*
AST (U/L)	41.1 ± 9.8	49.9 ± 12.0	368 ± 47.5*	316.4 ± 78.5*	112.0 ± 23.0*	90.7 ± 8.93	116.7 ± 44.4*	83.4 ± 14.4	183.7 ± 21.5*	221.4 ± 24.6*
GGT (U/L)	0.9 ± 0.1	1.0 ± 0.1	1.3 ± 0.2*	1.3 ± 0.3*	0.9 ± 0.1	0.9 ± 0.1	1.2 ± 0.1	1.0 ± 0.2	1.2 ± 0.2*	1.2 ± 0.9
ALP (U/L)	53.5 ± 2.7	59.7 ± 4.7	163.3 ± 18.8*	155.9 ± 11.1*	120.9 ± 12.4*	116.7 ± 10.5*	113.2 ± 5.6*	123.5 ± 20.8*	147.3 ± 17.8*	174.7 ± 14.3*

Values are mean ± SD (n=10 animals/group). CPA: Cyclophosphamide; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma glutamyltransferase; ALP: alkaline phosphatase. ANOVA followed by Tukey test. *p<0.05 compared to the negative control.

Table 2. Frequency of DNA damage (FD) evaluated by alkaline comet assay in bone marrow cells and hepatocytes of rats treated with aqueous extract of fruit of *Morinda citrifolia* (AEMC).

Group	Bone marrow (%)		Liver (%)	
	Male	Female	Male	Female
Negative control	12.1 ± 5.7	17.6 ± 3.2	19.1 ± 6.9	20.6 ± 9.5
CPA	29.8 ± 8.7*	74.6 ± 9.9*	62.8 ± 7.4*	73.8 ± 5.3*
AEMC 2.5 mg/kg	18.4 ± 8.3	12.1 ±7.3	41.6 ± 4.6*	26.1 ± 4.6
AEMC 5 mg/kg	44.6 ± 4.4*	26.8 ± 5.5	37.2 ± 5.8*	61.2 ± 4.1*
AEMC 10 mg/kg	78.4 ± 5.7*	68.1 ± 8.6*	78.1 ± 4.5*	70.4 ± 7.3*

Values are mean ± SD (n=10 animals/group). CPA: Cyclophosphamide 25 mg/kg. *p<0.05 compared to the negative control by ANOVA followed by Tukey test.

Induction of micronucleus formation

For complementary evaluation, the mutagenicity of AEMC was evaluated by the MN assay in bone marrow polychromatic erythrocytes and peripheral blood cells (Figure 4). Again, the doses 5 and 10 mg/kg showed a significant increase in MN (4.4 ± 0.8 and 7.8 ± 1.1; 7.4 ± 1.1 and 9.6 ± 1.4 for peripheral blood and bone marrow cells, respectively) (p<0.05). In addition to these results of mutagenicity by clastogenic and/or aneugenic effects, cytotoxicity was also evident, since the reduction in the PCE/NCE ratio was found in all doses tested, mainly at 10 mg/kg, which showed a significant decrease in PCE/NCE ratio for males (0.8 ± 0.1%) and females (1.0 ± 0.1%) (Figure 5).

DISCUSSION

Phytochemical analysis of methanolic extract of *M. citrifolia* indicated the presence of flavonoids, tannins, alkaloids, glycosides, saponins, carbohydrates, steroids and quercetin (Ramesh et al., 2012), corroborating our findings mainly in relation to the presence of flavonoids. Some reports describe products derived from *M. citrifolia*, especially those obtained from the fruit, has several pharmacological activities (Furusawa et al., 2003; Deng et al., 2007; Pachauri et al., 2012). Phenolic acids are consistently associated with reduced risk of cardiovascular disease, cancer and other chronic diseases by mechanisms

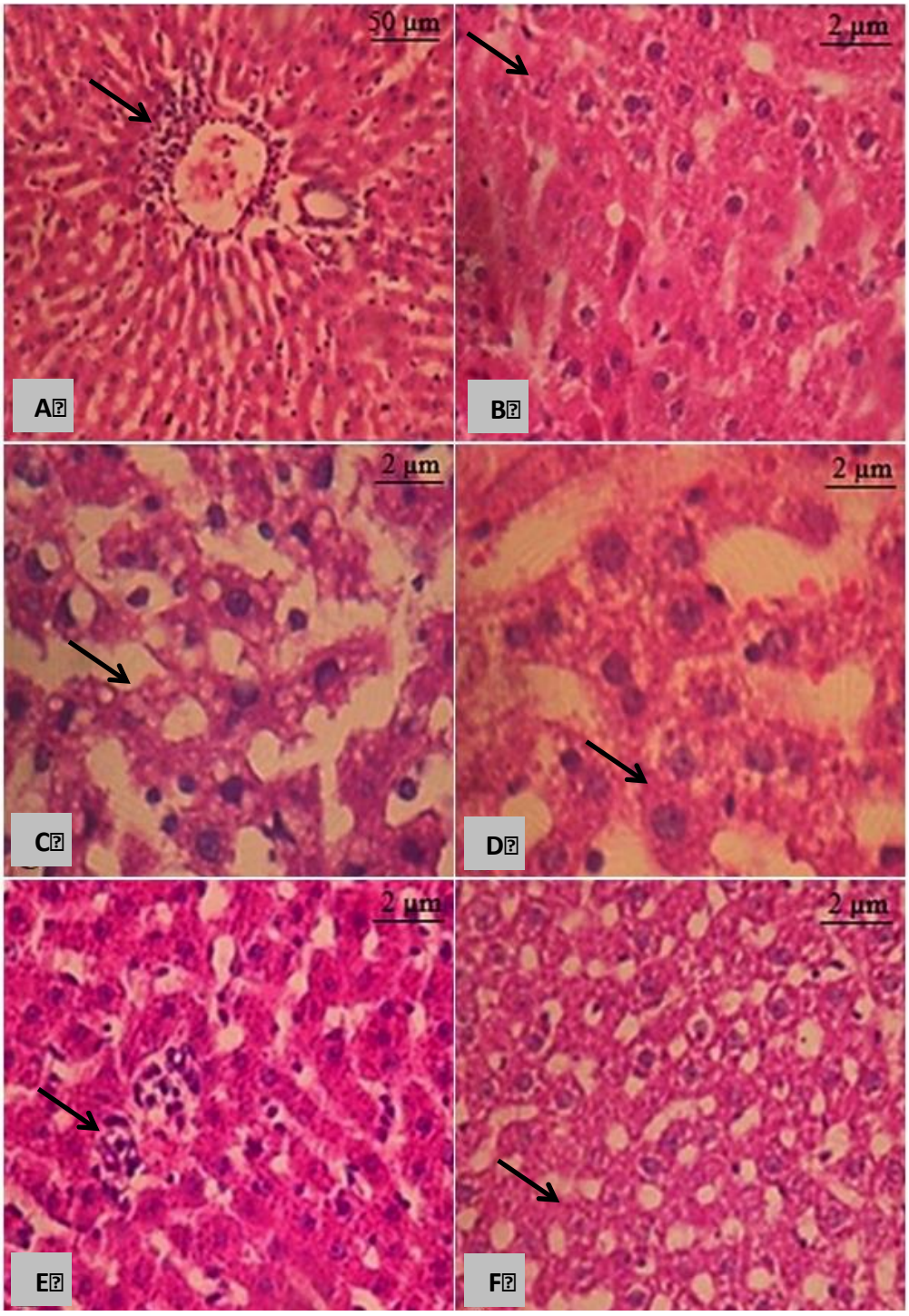


Figure 2. Liver histopathology analyzes of rats treated with aqueous extract of fruit of *Morinda citrifolia* (AEMC) at 10 mg/kg. A: Perivascular inflammatory infiltrate (200X). B: Nuclear fragmentation (400X). C: Microvacuolization (400X). D: Cellular swelling (400X). E: Lobular necroinflammatory (200X). F: Microvesicular steatosis (200X). Hematoxylin-Eosin staining.

related to the scavenging of free radicals and as prooxidant metals (antioxidant), although there are reports for toxic effects of compounds isolated from the fruit of M. citrifolia (Ee et al., 2009; Nualsanit et al., 2012; Aziz et al., 2014). Earlier reports indicated that fruits (Millonig et al., 2005; Stadlbauer et al., 2005; Yüce

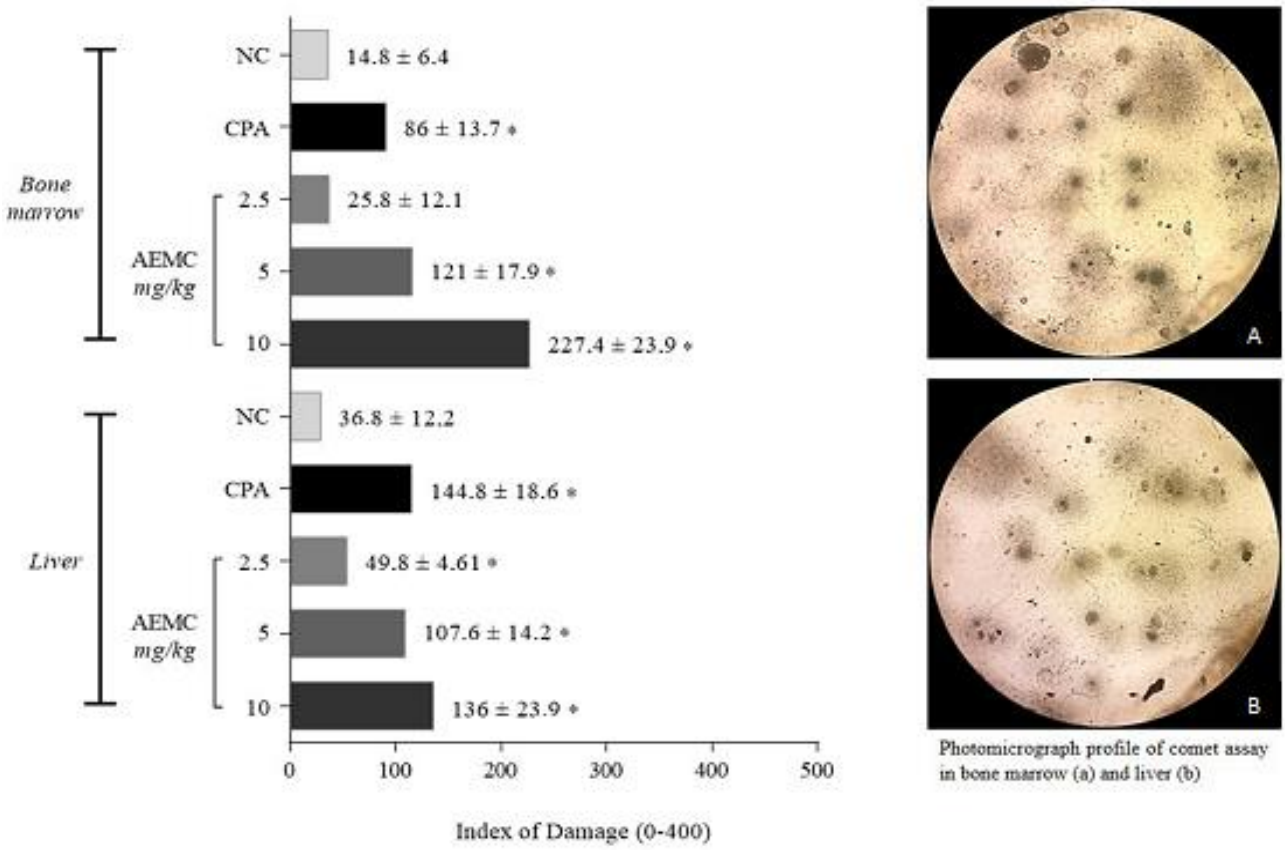


Figure 3. Genotoxicity in (A) bone marrow and (B) hepatic cells in rats treated with aqueous extract of fruit of *Morinda citrifolia* (AEMC). NC: negative control; CPA: cyclophosphamide 25 mg/kg. Values are mean ± S.D. (n=10 animals/group). *p<0.05 compared to the negative control by ANOVA followed by Tukey test.

et al., 2006), leaves (West et al., 2006; Lopez-Cepero et al., 2007) and juice (Sivagnanam et al., 2011; Mrzljak et al., 2013) of M. citrifolia are hepatotoxic in humans, since preparations for them are rich inanthraquinones and coumarins (scopoletin) (Ee et al., 2009), they induce generation of free radicals derived from oxygen and trigger oxidative stress. Free radicals cause depletion of intracellular reduced glutathione and mitochondrial membrane potential, thus initiating lipid peroxidation and eventually, cell death (Su et al., 2005; Bussmann et al., 2013).

Herein, using biochemistry and morphological analysis, it was shown that the AEMC at 2.5, 5 and 10 mg/kg induced hepatotoxicity in rats, as confirmed by levels of AST, ALT, ALP and GGT (this latter only at 10 mg/kg). Levels of ALT, AST and ALP were found to be approximately 2 to 6-fold higher in *M. citrifolia* treated group than those seen in untreated animals. Hepatic ‘‘leakage’’ enzymes are usually cytosolic, which gain access into circulation by leaking out of the cytoplasm either by reversible (membrane blebs) or irreversible (mitochondrial membrane damage) hepatic injury (Gores et al., 1990; Van Hoof et al., 1997). Following acute injury resulting in moderate to severe zonal necrosis by a liver toxicant, there is generally a moderate to marked increase in the serum ALT and AST activities which returns to normal within a few days indicative of resolution of injury. Although signals of tissue impairment have also been seen, since even necrosis are found with conjunctive tissue preservation, liver always presents good regeneration capacity, generally achieving a complete hepatic restoration (Hall and Cash, 2012). Although our studies have linked *M. citrifolia* consumption with liver damage, some assessments suggest it may protect liver from CCl_4-induced damage (Wang et al., 2008).

Toxicity can also be evidenced in genetic materials by *in vivo* Comet assay, a technique with applicability in different tissues and able to detect low levels of DNA damage in a small number of cells (Pourrut et al., 2015). Using this method in its alkaline version, it was possible to detect DNA single and double-strand breaks in the liver and bone marrow mononuclear murine cells, especially at AEMC 10 mg/kg. These genotoxic effects have dramatic effects on higher-order chromatin structure because of its supercoiling and tight packaging within the

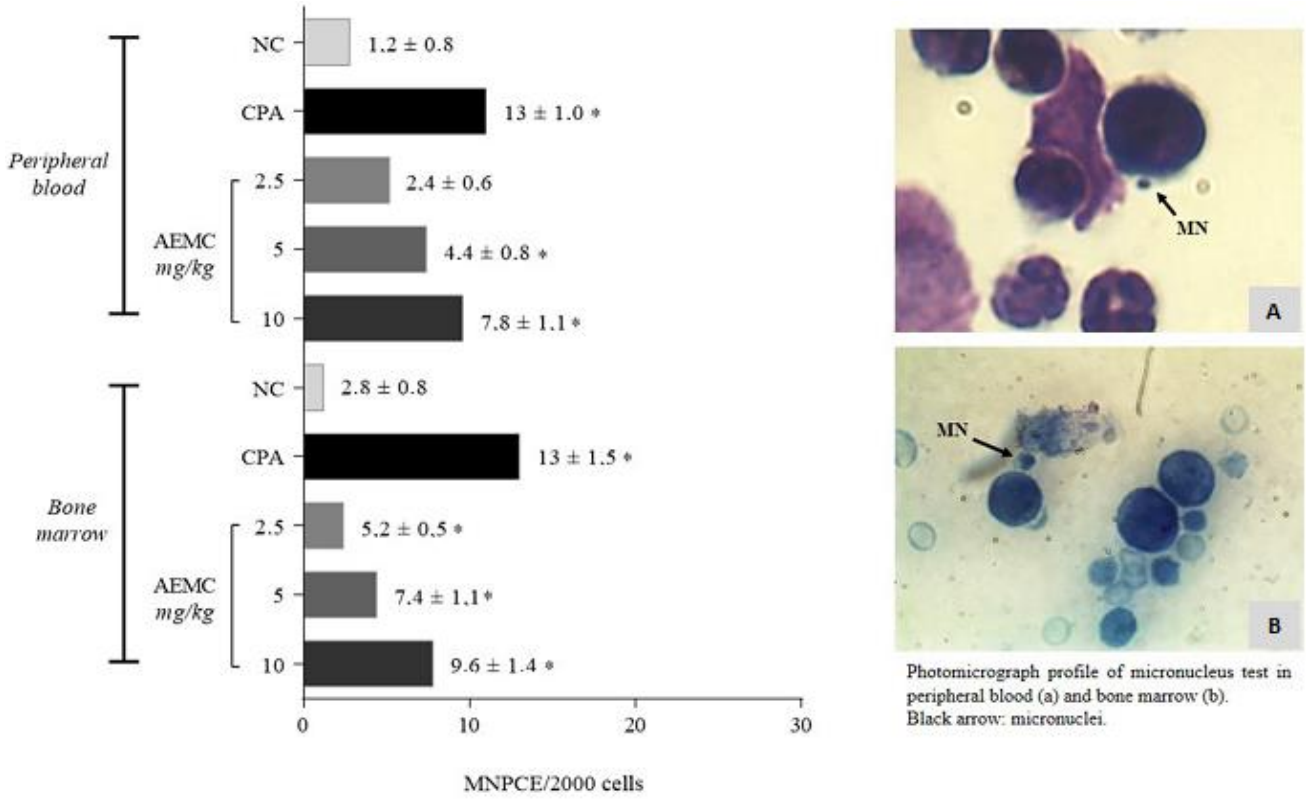
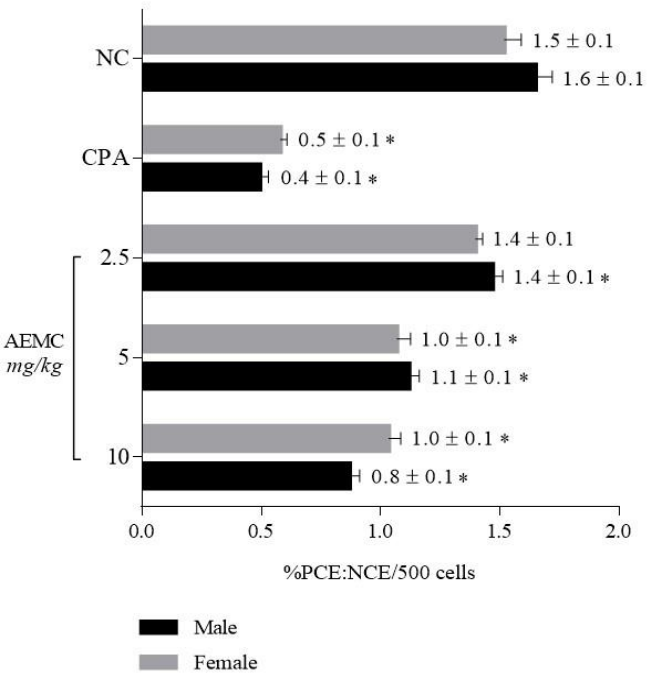


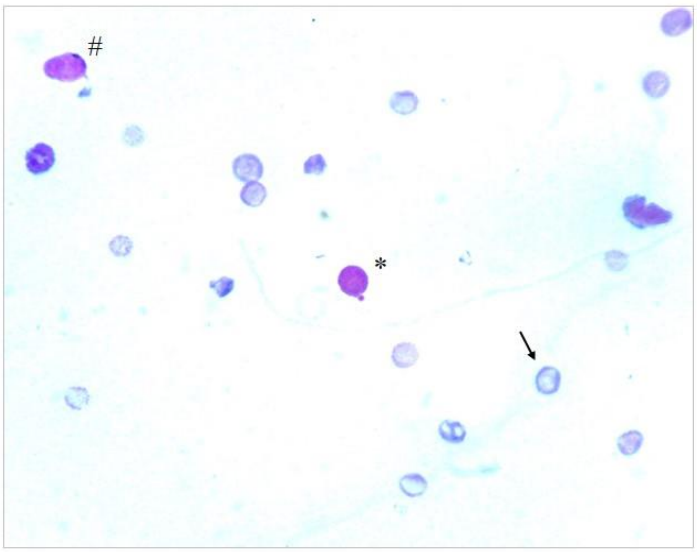
Figure 4. Mutagenicity in (A) bone marrow and (B) peripheral blood cells of rats treated with aqueous extract of fruit of *Morinda citrifolia* (AEMC). NC: Negative control; CPA: cyclophosphamide 25 mg/kg; MN: micronuclei; MNPCE: polychromatic erythrocytes with micronuclei. Values are mean ± S.D. (n=10 animals/group). *p<0.05 compared to the negative control by ANOVA followed by Tukey test.

nucleus (Fairbairn et al., 1995).

It should be emphasized that genotoxic damages can be repaired (Azqueta et al., 2014). However, extensive genotoxic injuries probably led to unrepaired genoma. This probably occurred at higher doses of AEMC, since chromosome breakage, chromosome rearrangement, chromosome loss, non-disjunction, gene amplification, necrosis and apoptosis, resulting in micronuclei, mainly from chromosome breaks or whole chromosomes that fail to engage with the mitotic spindle when the cell divides (Fenech et al., 2011). Notably, studies confirm that the high frequency of MNs increases the risk of developing tumors, because cancer is based on the accumulation of mutations on genetic material (Cardinale et al., 2012; Bhatia and Kumar, 2013).

Genotoxicity and mutagenicity findings can be attributed to the effects of complex mixtures of bioactive compounds present in *M. citrifolia* fruits. One of its components, caffeic acid (CA), has been related to induction of cell cycle arrest and apoptosis, protein kinases changes and inhibition of cyclooxygenase-2 (COX-2) activity (Kuo et al., 2015) and can generate hydrogen peroxide and hydroxyl radicals (Ruiz-Laguna and Pueyo, 1999). Ellagic acid (EA) has anticarcinogenic DNA injury and micronuclei figures were more usually found. In such condition, when a damage of superior intensity leads to unrepaired genomic instability at the chromosome/molecular level and such defects accumulate point mutations in the DNA, it can reflect on effects due to inhibition of tumor proliferation and angiogenesis, as well as induction of breaking connections between DNA caused by carcinogens (Zhang et al., 2014). Rutin possesses antitumor activity by inducing DNA damage to the mutant cell BRCAgene, probably by cell cycle arrest in G2/M phase and apoptotic effects on neuroblastoma cell lines (Chen et al., 2013; Maeda et al., 2014). However, this flavonoid can contribute to the growth of peritoneal tumors by the inhibition of macrophage migration in metastatic processes (Van der Bij et al., 2008). So, despite the pharmacological properties of these compounds, they probably have low selectivity on tumor cells and such compounds also attack normal tissues, which can explain, at least in part, the hepatotoxicity and mielotoxicity with AEMC. It is likely that distinct bioactive substances may jointly or independently contribute to nonselective biological effects (Ferreira et al., 2014). Drug/chemical-mediated hepatic injury is the most





Photomicrograph profile of bone marrow. Asterisk: MNPCE. Hash: PCE. Black arrow: NCE.

Figure 5. Cytotoxicity in bone marrow cells of rats treated with aqueous extract of fruit of *Morinda citrifolia* (AEMC). NC: negative control. CPA: Cyclophosphamide 25 mg/kg. MN: micronuclei; NCE: normochromatic erythrocyte; PCE: polychromatic erythrocytes; MNPCE: polychromatic erythrocytes with micronuclei. Values are mean ± SD (n=10 animals/group). *p<0.05 compared to the negative control by ANOVA followed by Tukey test.

common manifestation of drug toxicity and accounts for greater than 50% of acute liver failure cases. Hepatic damage is the largest obstacle to the development of drugs and is the major reason for withdrawal of drugs from the market (Cullen and Miller, 2006).

Conclusion

Outcomes pointed that the AEMC, at 5 and 10 mg/kg, predominantly, induces hepatotoxicity, genotoxicity and mutagenicity in liver, bone marrow and peripheral blood cells of rats. These findings suggest clastogenic and/or aneugenic effects and genetic instability activated by *M. citrifolia*, which indicates precaution regarding the consumption of medicinal formulations or folk preparations based on this plant.

Conflict of Interests

The authors have not declared any conflict of interests.

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