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Research Article

Evaluation of Cytotoxicity, Acute Toxicity, Genotoxicity, Mutagenic and Antimutagenic Potential of *Elaeocarpus Serratus L.* Fruit Extract

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ABSTRACT

Elaeocarpus serratus L. (Ceylon olive) fruits in popular medicine are used in the treatment of dysentery and diarrhea. Despite the properties of the fruit, there are no records in Brazil of studies that validate its safe use. The study aimed to evaluate the cytotoxicity, acute toxicity, genotoxicity, mutagenic, and antimutagenic potential of the aqueous extract of the fruits of *E. serratus* (AEES) through the platelet viability assay (MTT assay), acute toxicity test, micronucleus test and comet assay in rodents, and through the SMART assay with *Drosophila melanogaster*. It has shown moderate cytotoxic properties, the estimated LD50 of the AEES is greater than 2000 mg/kg and has no genotoxic potential in peripheral blood cells or clastogenic and aneugenic activity in cells from the bone marrow of rats. It is not a mutagenic agent and presents antimutagenic activity, attested by SMART assay.

Keywords: Acute toxicity; Genotoxicity; Cytotoxicity; Antimutagenic

Abbreviations: AEES: Atomic Energy Education Society; MTT assay: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; SMART assay: Self-Monitoring Analysis and Reporting Technology

INTRODUCTION

Since ancient times, the use of medicinal plants has represented the only therapeutic resource of diverse ethnic groups and communities, since it summarizes knowledge, practices, and skills in the diagnosis, treatment, and prevention of diseases, preserving the cultural uniqueness of each people [1,2]. The use of medicinal plants still prevails in developing countries, which drives the investigation of their characteristics and therapeutic properties for the development and improvement of phytotherapeutics [3]. The genus Elaeocarpus, derived from the Greek word "elaia" meaning olive and "karpos" meaning fruit, belongs to the *Elaeocarpaceae* family, which includes 10 genera and 400 species of tropical trees [4]. Among the several species, the *Elaeocarpus serratus* L. stands out. Originally from India, known as Ceylon Olive, it is a medium-sized tree, with simple leaves and fruit considered drupes, widely distributed from East Africa until Brazil. With a sweet and slightly sour taste, the *E. serratus* fruit is popularly eaten in the form of snacks in Sri Lanka and companies are manufacturing canned fruit as an alternative to the traditional olive [5]. In popular medicine, the species is used as a diuretic and cardiovascular stimulant. The decoction of the peel is used in the treatment of hemorrhages and ulcers, the leaves in the treatment of rheumatism and as an antidote for poisoning, while the juice of the fruits is used in the treatment of dysentery and diarrhea, in addition to stimulating the appetite of hospitalized patients [6]. Clinical trials with the fruits of *E. serratus* report a potent anti-diabetic effect in association with streptozotocin in vivo and in vitro models [7,8], and the species has antimicrobial and anti-inflammatory activities [9,10]. Despite the properties of *E. serratus* fruits, toxicity studies proving the safe use of the fruit are scarce in Brazil. Therefore, the research aimed to evaluate the cytotoxicity, acute toxicity, genotoxic, mutagenic, and antimutagenic potential of the aqueous extract of the fruits of *E. serratus* through the platelet viability assay (MTT assay), acute toxicity test, micronucleus test and comet assay in rodents and the SMART test with *Drosophila melanogaster*.

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METHODOLOGY

Preparation of the extract

The species is registered at Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado of Brazil (Registration Number AA46584). The *E. serratus* L. fruits were collected in Dourados-Mato Grosso do Sul, Brazil (22°09'16.3 "S54°48'17.8 "W) in the mature stage (average width between 12.31 ± 1.05 mm) [11] and selected to obtain a uniform lot according to size and absence of lesions. The material was washed and sanitized with a 0.66% solution of sodium dichloroisocyanurate dihydrate (3% active chlorine content). The fruits were peeled, pulped, separated without seeds, and stored at -5°C until the extract was prepared.

The fruit of *E. serratus* L. was dried in air circulation at 40°C. After drying, the material was placed in a semi-industrial processor until a powder texture was obtained and stored in a dry environment at 25°C. The aqueous extract of the *E. serratus* L. fruit Atomic Energy Education Society (AEES) was prepared by mixing the dry material in powder with distilled water until full homogenization, then left in agitation under light for three days at 25°C. After three days, the obtained filtrate was frozen at -80°C and lyophilized. The lyophilized extract was diluted in the vehicle designated for the study and used in the experiments.

Characterisation of the extract

The chemical composition of the aqueous extract of the fruits of *Elaeocarpus serratus* L. was performed by Freitas de Lima et al. [12]. The main findings of the chemical analysis of the fruits highlights the presence of flavonoids [120.49 mg of QE.100 g^{-1}], condensed tannins [16142.40 mg of CE.100 g-1], carotenoids [4.97 mg.100 g^{-1}] and vitamin C [5.93 mg.100 mg.g^{-1}]. By High-performance Liquid Chromatography (HPLC) method, the presence of quercetin [12.9 mg.g^{-1}] and kaempferol [13.4 mg.g^{-1}] was observed, while by Gas and Liquid Chromatography (GC-MS) method, β-amyrin (12.5 mg.g^{-1}), α-amyrin (4.0 mg.g^{-1}), β-amyrin acetate (4.3 mg.g^{-1}) and stigmasterol (3.6 mg.g^{-1}), with α-amyrin and β-amyrin being most prevalent in the fruit pulp of *E. serratus* L [12].

Animals and treatments

Seventy Wistar rats (*Rattus novergicus*) of both sexes (30 males and 40 females), aged 45 to 60 days were used in the experiments. The animals were supplied by the Federal University of Grande Dourados (UFGD) Central Biotery and kept in rodent cages (434 × 16 cm), with 5 animals per cage, under conditions of temperature (22 ± 2°C) and humidity (40-60%), and with ad libitum access to water and food. The experimental procedures were conducted following the ethical precepts for the use of animals in research and approved by the Ethics Committee for the Use of Animals of UFGD (protocol n. 24/2018.2).

Two groups of females (n=5 each) were established for acute toxicity, the first treated with a single dose of the vehicle (1 ml of ultrapure water Milli-Q®+Tween-80®) via oral gavage and the second treated with a single dose of 2000 mg/kg of AEES associated with 1 ml of ultrapure water Milli-Q®+Tween 80® via oral gavage. Six groups of 5 males and 5 females were established for the genotoxicity tests, as follows: (1) Naive group, treated for 28 days orally with 1 ml of ultrapure water Milli-Q®; (2) groups treated for 28 days with the AEES orally (125, 250, 500 and 1000 mg/kg associated 'with 1 ml of ultrapure water Milli-Q®+Tween-80®); (3) positive control group, treated with 20 mg/kg bodyweight of cyclophosphamide monohydrate intraperitoneally 24 hours before euthanasia.

Platelet viability assay (MTT assay)

The MTT assay was performed according to the methodology presented by Mosmann [13]. Using 96-well plates, platelets (1.5 × 10^8 platelets/ml) were incubated with AEES (0.03, 0.12, 0.3, 1, 3, and 10 µg/ml^{-1}) for 5, 15 and 30 minutes. The positive control was 10% triton-X. After the incubation period, platelets were incubated with 5 mg/ml of MTT solution for 3 hours in a CO_2 incubator. Then MTT dye was removed and 100 ml of solubilization solution (SDS 10%acidified) were added to the wells. Absorbance was measured at 540 nm using a microplate reader (Synergy TM H1 Hybrid Reader, BioTek, United States).

Acute toxicity

Acute toxicity was evaluated following the Organisation for Economic Cooperation and Development guidelines [14]. After administration, the animals were observed especially during the first 24 hours, at 15, 30, and 60 minutes and every 4 hours, and then once a day for 14 consecutive days.

During the study period, toxic general signs were observed according to hippocratic screening [15], and the following parameters were evaluated: bodyweight variation, clinical signs (piloting, contortions, tremors, convulsions, cyanosis, ataxia, diarrhea), and behavioral changes. Physiological data were observed and recorded daily (body weight, water, and food consumption). Mortality was observed so that the lethal dose for 50% of the animals (LD50) could be estimated.

The animals were euthanized on day 15 with isoflurane overdose until no corneal reflex. After confirmation of death by analysis of vital signs and corneal reflex, the vital organs (heart, lung, liver, right kidney, spleen, right ovary, and uterus) were collected and analyzed. Observing any alteration in the general aspect, color and texture of the organs, the histopathological analysis was performed.

Comet assay

The comet assay was conducted from peripheral blood analysis and followed the Organisation for Economic Co-operation and Development (OECD) guidelines [16]. Before euthanasia, each animal was anesthetized with an association of ketamine and xylazine and a small cut was made at the tip of the tail for blood collection and allocated on slides with agarose. Then, the slides remained in lysis for 2 hours to remove the cellular membrane and extravasation of the DNA. The slides with the extravasated DNA were placed in electrophoresis equipment, soaked in alkaline solution, and submitted to electrophoretic run for 20 minutes at a temperature of -4°C and voltage of 25 V and 300 mA. After the run, the slides were allocated in a neutralization solution in 3 cycles of 5 minutes each. Then, they were fixed with absolute alcohol for 10 minutes and cooled under the light. For the analysis, the slides were stained with ethidium bromide (0.002 mg/ml) and analyzed with a fluorescence microscope with an excitation filter (420-490 nm) and barrier filter (520 nm).

An analysis of 100 cells per animal was performed. The size of the comet formed was classified in a score from 0 to 3, where 0 indicates the absence of DNA fragments; 1 indicates tail smaller than the nucleoid; 2 indicates tail with a diameter equal to or up to twice the diameter of the nucleoid; 3 indicates tail with diameter three times the diameter of the nucleoid [17]. The type and size of the comet

formed in each sample were considered to assess genotoxic damage.

Micronucleus test

The micronucleus test was conducted following the Organisation for Economic Co-operation and Development (OECD) guidelines [18]. The analysis of polychromatic erythrocytes was performed from cells obtained from the bone marrow of animals treated for 28 consecutive days.

After euthanasia, the right femur of each animal was removed and separated from adjacent muscles. The two extremities of the femur were cut, and the bone marrow was washed with 1 ml of bovine fetal serum and immediately allocated in a microtube for centrifugation. After 5 minutes of centrifugation at 1000 rpm, the supernatant was discarded, and the pellet was used to make the slides. The slides were fixed in methanol for 10 minutes and stained with Giemsa for 15 minutes. For each sample, 2000 erythrocytes were analyzed to identify micronucleated polychromatic erythrocytes, an indicator of genotoxic damage.

The ratio of Normo Chromatic Erythrocytes (NCE)/Poly Chromatic Erythrocytes (PCE) was also performed, which is a cytotoxic assay that evaluates if the test substance causes toxic effects on bone marrow erythrocytes. To calculate the NCE/PCE ratio 200 erythrocytes were randomly counted in the same macroscopic field, and the indication of cytotoxic damage is when the ratio is close to 0 and close to 1 when there is no cytotoxic damage [19].

Somatic Mutation and Recombination Test (SMART Test)

Three strains of *Drosophila melanogaster* were used: [1] the mwh lineage (multiple wing hairs), homozygous, with the genetic constitution mwh/mwh, characterized by the presence of multiple hairs [2] the flare 3 lineage (flr³), presenting the allele flr³ in hemozygosis, with the genetic constitution flr³/In TM³ Bds and cut edge wings; [3] Oregon flare³ (ORR flr³), which has chromosomes 1 and 2 of Oregon R (resistant to DDT) and genes that allow a high level of bio activation with the enzymes of cytochrome P450, with the genetic constitution ORR;flr3/In(3LR)TM3, ri pp sep l(3)89Aa bx34e and a BdS [20,21].

Two crossings were performed: standard crossings (ST-standard) between males mwh and virgin flr³ females, and high bioactivation crossings (HB-high bioactivation) between males mwh and ORR flr³ virgin females. Eggs from each crossing were collected after 8 hours in culture flasks containing an agar-agar base (0.04 g/ml), biological yeast and supplemented with sugar. The larvae that hatched within 72 hours were washed with tap water and collected with a sieve. The groups of larvae from each crossing were transferred to identified glass jars, based on an alternative medium consisting of 1.5 g of industrialized mashed potato flakes (Yoki®), following the following protocols (mutagenicity and antimutagenicity, respectively): (1) three concentrations of the AEES (1.25, 2.5 and 5.0 mg/ml associated with distilled water and Tween-80®); [2] three concentrations of the AEES (1.25, 2.5 and 5.0 mg/ml associated with distilled water and Tween-80®) in association with doxorubicin hydrochloride (DXR) (Adriblastina®RD 10 mg, Pfizer Laboratories Ltd, in powder mixed with distilled water until reaching the dose of 0.125 mg/ml). The negative control of both protocols received vehicle (3% ethanol, 1% Tween-80®, and distilled water).

Adult flies presented genotype mwh+/flr³⁺ (trans-heterozygous marked-MH) and mwh+/+TM3, BdS (heterozygous balanced-BH), were collected and fixed in 70% alcohol. The wings were fixed on glass slides with Faure solution (30 g of Arabic gum, 50 g of chloral hydrate, 20 ml of glycerol and 50 ml of water) and analyzed by light microscopy, with 400x magnification, to identify the types of mutations. All wings presented the heterozygous marker mwh/flr³, being possible to observe three types of stains: simple small (one to two stains), simple large (two or more stains) and twin stains (both subclones mwh and flr³).

Statistical analysis

The results of MTT assay, acute toxicity, micronucleus test, and comet assay were expressed as mean ± standard deviation for each treatment group. The statistical evaluation for the micronucleus test and comet assay was performed by analysis of variance (ANOVA) followed by the Tukey test, considering the value of $p<0.05$ to consider significant differences between the treated and control groups. The GraphPad Prism 8® software was used to make the graphs.

For the statistical analysis of the SMART test, the Frei and Wrügler [22] method was used, where the findings were distinguished by the type and size of mutant stains and analyzed by a bi-caudal chi-square test for the proportions, with a significance level of $\alpha=\beta$ 0.05, where the statistical diagnosis was positive (+), negative (-) or inconclusive (i). 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 doxorubicin, according to Abraham (21): %R=DXR mutation frequency-mutation frequency of the samples associated with DXR × 100/ DXR mutation frequency.

RESULTS

MTT assay

Figure 1 shows the results of the MTT assay. In lower concentrations (0.03, 0.12 and 0.3 μg.ml^{-1}) the AEES did not decrease the platelet viability during the 5, 15 and 30 minutes time. The concentrations 3 μg.ml^{-1} and 10 μg.ml^{-1} of AEES showed significant statistical differences when compared to the baseline and positive group (Triton-X), decreasing the platelet viability during the time of the assay.

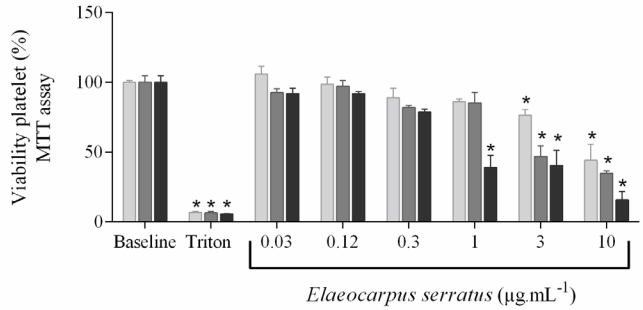

Figure 1: The results of the MTT assay. Note: ( ) 5 min; ( ) 10 min; ( ) 15 min

Acute toxicity

After exposure to the 2000 mg/kg dose of AEES, there was no mortality among the animals studied. Females treated with the AEES showed no clinical signs of toxicity, nor did behavioral changes due to hypocratic screening throughout the study period. The mean weight ranged from 187.50 g ± 11.47 for the control group to 186.60 g ± 15.95 for the treated group. There were no significant changes in weight gain and water and food consumption between groups (Table 1).

Table 1: Values expressed in mean ± standard deviation. N=5 animals I group for acute toxicity and n=10 animals I group for subacute toxicity. "P<0.05 (ANOVA I Tukey) compared with the control group.

Acute toxicity		
	Control	2000 mg/kg
Female		
Initial weight (g)	178.00 ± 18.55	178.00 ± 18.55
Final weight (g)	187.50 ± 11.47	186.60 ± 15.95
Body weight gain (%) Food intake (g/day)	21.94 ± 4.33	18.32 ± 1.47
	64.31 ± 12.85	71.69 ± 19.32
Water intake (mUday)	151.82 ± 47.92	172.00 ± 65.12
Liver (g)	3.80 ± 0.32	3.35 ± 0.32
Kidney (g)	0.40 ± 0.02	0.37 ± 0.02
Spleen (g)	0.24 ± 0.01	0.22 ± 0.03
Heart (g)	0.30 ± 0.02	0.30 ± 0.02
Lung (g)	0.59 ± 0.07	0.57 ± 0.08
Ovary (g)	0.03 ± 0.00	0.02 ± 0.00
Uterus (g)	0.20 ± 0.08	0.28 ± 0.13

Comet assay

The average weight of the animals ranged from 150.40 g ± 11.93 for females to 256.5 g ± 25.94 for males. Only in the positive control group were there significant statistical differences in the number of damaged cells and the final score of damage (Figures 2 and 3), and in the Naive group and those treated with AEES, there were no significant statistical differences ($p \leq 0.05$) (Table 2).

Micronucleus test

All groups of both sexes treated with AEES presented a lower frequency of micronuclei when compared with the positive control, without significant statistical differences between them (Figure 2).

Table 3 presents the ratio of polychromatic erythrocytes and normochromatic erythrocytes (PCE/NCE), where only statistical differences were observed in the positive control group of both genders (0.19 ± 0.04-females; 0.20 ± 0.02-males), showing that this group did not present a cytotoxic effect, different from the doses of the AEES, which presented values close to 1 (Figure 3).

Smart test

On Table 4, the negative control showed a frequency in the total of mutant stains of 0.30 in the descendants of the ST crossing and 0.50 in the descendants of the HB crossing, while the frequency of stains between the doses of the AEES ranged from 0.10 to 0.35 in the ST crossing and from 0.20 to 0.80 in the HB crossing, showing no statistical differences when compared with the negative control.

As for Table 5, the negative control showed a frequency of formation of mutant stains of 0.30 and 0.50, referring to the two crossings, respectively. With the positive control, the frequency of mutant stains was 2.60 at the ST crossing and 6.75 at the HB crossing. Comparing the treated individuals with the concentrations of the AEES at the ST crossing, with those treated with DXR, the frequency of mutant staining ranged from 1.55 to 4.35, with a reduction of 67.30% and 94.20%, respectively for concentrations of 2.5 and 5.0 mg/ml of AEES. At the HB crossing, the frequency of mutant stains between the groups ratted with the AEES ranged from 2.55 to 3.65, with a reduction of damage caused by DXR ranging from 45.90% to 62.20% (Tables 4 and 5).

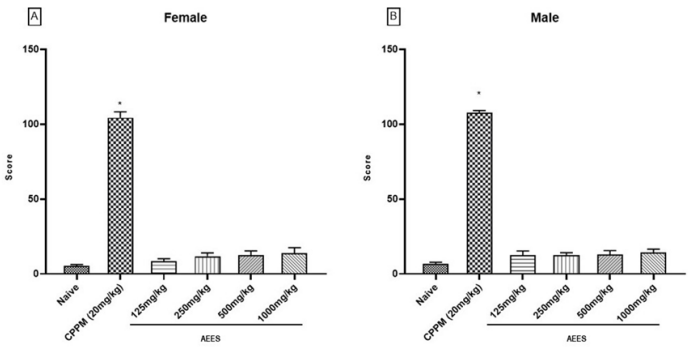

Figure 2: Effects of the treatment with the aqueous extract of *Elaeocarpus serratus* fruit (AEES) and cyclophosphamide (CPPM) (positive control) in the final score of DNA damage of wistar rats. Note: *Significant statistical difference (p<0.05; ANOVA/Tukey).

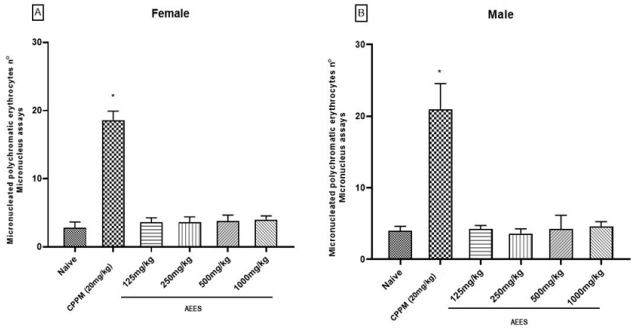

Figure 3: Effects of the treatment with the aqueous extract of *Elaeocarpus serratus* fruit (AEES) and cyclophosphamide (CPPM) (positive control) in the micronucleated polychromatic erythrocites count of wistar rats bone marrow. Note: *Significant statistical differeces (p<0.05;ANOVA/Tukey).

Table 2: Frequency of damage by the comet assay in rats treated orally with aqueous extract of *Elaeocarpus serratus* fruit.

Groups	Injured cells	Classes of damage				Score
Female		0	1	2	3	
Naive	4.60 ± 0.68[a]	95.40 ± 0.63	4.00 ± 0.45	0.60 ± 0.40	0.00 ± 0.00	5.20 ± 1.02
Cyclophosphamide(CPPM)	66.80 ± 1.56[b]	33.20 ± 1.68	34.00 ± 2.02	28.00 ± 1.51	4.80 ± 1.24	104.40 ± 3.94[a]
AEES 125 mg/kg	7.20 ± 0.80[a]	92.80 ± 0.91	6.00 ± 0.77	1.00 ± 0.55	0.20 ± 0.20	8.60 ± 1.50
A EES 250 mg/kg	8.20 ± 1.07[a]	91.80 ± 1.07	5.60 ± 0.87	1.60 ± 0.68	1.00 ± 0.63	11.80 ± 2.22
AEES 500 mg/kg	6.80 ± 1.16[a]	93.20 ± 1.16	3.40 ± 1.08	1.20 ± 0.97	2.20 ± 0.91	12.40 ± 2.97
AEES 1000 mg/kg	8.20 ± 1.59[a]	91.80 ± 1.59	4.60 ± 1.43	1.60 ± 0.98	2.00 ± 0.95	13.80 ± 3.73
Male						
Naive	6.20 ± 0.58[a]	93.80 ± 0.58	5.60 ± 0.40	0.60 ± 0.40	0.00 ± 0.00	6.80 ± 0.91
Cyclophosphamide(CPPM)	69.40 ± 1.21[b]	30.60 ± 1.21	34.00 ± 1.14	32.60 ± 1.63	2.80 ± 0.97	107.60 ± 1.60[a]
AEES 125 mg/kg	9.20 ± 1.24[a]	90.80 ± 1.24	6.60 ± 0.51	1.80 ± 0.73	0.80 ± 0.58	12.60 ± 2.71
A EES 250 mg/kg	8.80 ± 0.58[a]	91.20 ± 0.58	6.20 ± 0.73	1.60 ± 0.75	1.00 ± 0.45	12.40 ± 1.80
AEES 500 mg/kg	9.40 ± 1.36[a]	90.60 ± 1.36	6.80 ± 0.37	1.80 ± 0.91	0.80 ± 0.37	12.80 ± 2.71
AEES 1000 mg/kg	9.80 ± 0.86[a]	90.20 ± 0.86	6.00 ± 0.45	3.00 ± 1.14	0.80 ± 0.37	14.40 ± 2.04

Note: Values expressed as mean ± SEM, n=10 animals/group. Different letters indicate statistically significant differences ($p < 0.05$; ANOVA/Tukey). A-Significantly different from negative and treatment groups. Class 0-no damage;class 1-tailof comet shorter than the diameter of nucleoid; Class 2-tail of comet once or twice the diameter of nucleoid; Class 3-tail of comet more than twice the diameter of nucleoid. **Score:** Type of damage x number of cells with damage.

Table 3: Mean frequency ± SEM of the micronucleated polychromatic erythrocytes (n°) and the ratio between polychromatic erythrocytes/normochromatic erythrocytes-PCE/NCE-micronucleus assay.

Experimentalgroups	Micronucleus/ Mean frequency ± SEM		PCE/NCE ± SEM	
	Female	Male	Female	Male
Naive	2.80 ± 0.86	4.00 ± 0.63	0.59 ± 0.06	0.57 ± 0.06
CPPM	18.60 ± 1.32*	21.00 ± 3.56*	0.19 ± 0.04*	0.20 ± 0.02*
AEES 125 mg/kg	3.60 ± 0.68	4.25 ± 0.48	0.62 ± 0.03	0.55 ± 0.06
AEES 250 mg/kg	3.60 ± 0.81	3.60 ± 0.68	0.59 ± 0.05	0.57 ± 0.10
AEES 500 mg/kg	3.80 ± 0.86	4.25 ± 1.93	0.55 ± 0.05	0.55 ± 0.16
AEES 1000 mg/kg	4.00 ± 0.55	4.60 ± 0.68	0.54 ± 0.03	0.58 ± 0.08

Note: * Statistically significant differences ($p<0.05$); ANOVA/Tukey

Table 4: Frequency of mutant stains observed on the wings of the descendants of *Drosophila melanogaster* from standard (ST) and high bio activation crossings treated with aqueous extract of *Elaeocarpus serratus* fruit

Genotypes and Treatment (mg/ml)	N.° of ind.	Stains per individual (n°of stains) statystical diag.				Total of mwh stains[c]
		SSS (1-2cels)[b] m=2	SLS (>2cels)[b] m=5	TS m=5	TS m=2	
mwh flr³l ST						
Negat ve Control	20	0.25 (05)	0.05 (01)	0.00 (00)	0.30 (06)	6
AEES [1,25]	20	0.10 (02)-	0.00 (00) i	0.00 (00) i	0.10 (02)-	2
AEES [2,5]	20	0.15 (03) i	0.05 (01) i	0.00 (00) i	0.20 (04) i	2
AEES [5,0]	20	0.15 (03) i	0.20 (04) i	0.00 (00) i	0.35 (07) i	7
mwh flr³l HB		0.54 ± 0.03	0.54 ± 0.03	0.54 ± 0.03	0.54 ± 0.03	0.54 ± 0.03
Negative Control	20	0.30 (06)	0.20 (04)	0.00 (00)	0.50 (10)	10
AEES [1,25]	20	0.40 (08) i	0.15 (03) i	0.05 (01) i	0.60 (12) i	5
AEES [2,5]	20	0.20 (04) i	0.00 (00) -	0.00 (00) i	0.20 (08)-	4
AEES [5,0]	20	0.10 (02)-	0.70 (14)+	0.00 (00) i	0.80 (16) i	15

Note: [a]Statistical diagnosis according to Frei and Wurgler (1988); +: Positive; -: Negative; i: inconclusive; M: Multiplication factor to evaluate significant negative results. blncluding simple rare flr³ stains; [C] Considering *mwh* clones for simple stains and mwh for twin stains; SSS: Simple Small Stains; SLS: Simple Large Stains; TS: Twin Stains; TS: Total of Stains

Table 5: Frequency of mutant stains observed on the wings of the descendants of *Drosophila melanogaster* from standard (ST) and high bioactivation crossings treated with aqueous extract of *Elaeocarpus serratus* fruit associated with Doxorubicin.

Genotypes and treatment (mg/ml)-	N.° of individuals	Strain per individual(n° of strain) statistical diag.[a]				Total of mwh stainsc SSS (1-2cels)[b] m=2	Stains per individual(n°of stains) statistical diag.aSLS (>2cels) [b] m=5
		SSS (1-2cels)[b] m=2	SLS (>2cels)[b] m=5	SSS (1-2cels)[b] m=2	SLS (>2cels)[b] m=5		
Mwh/flr^3/ST							
Negative control	20	0.25 (05)	0.05 (01)	0.00 (00)	0.30 (06)	6	
Positive control DXR	20	0.50 (10) i	1.45 (29)+	0.65 (13)+	2.60 (52)+	48	
EAES [1,25]+DXR	20	0.90 (18) i	1.55 (31)-	0.60 (12) i	3.05 (61)-	54	17.30 %
EAES [2,5]+DXR	20	1.70 (34)+	1.65 (33)-	1.00 (20) i	4.35 (87)+	69	67.30 %
EAES [5,0]+DXR	20	0.30 (06) i	0.75 (15) +	0.50 (10) i	1.55 (31)+	24	94.20 %
mwh/flr3// HB							
Negative control	20	0.30 (06)	0.20 (04)	0.00 (00)	0.50 (10)	10	
Positive control DXR	20	2.40 (48)+	2.55 (51)+	1.80 (36) +	6.75 (135)+	124	
EAES [1,25]+DXR	20	0.60 (12)+	1.35 (27)+	0.60 (12)+	2.55 (51)+	43	62.20%↓
EAES [2,5]+DXR	20	1.10 (22)+	1.20 (24)+	0.90 (18)+	3.20 (64)+	54	52.50% ↓
EAES [5.0]+DXR	20	1.20 (24)+	1.85 (37) i	0.60 (12) +	3.65 (73)+	54	45.90% ↓

Note: [a]Statistical diagnosis according to Frei and Wurgler (1988); +:positive; -: negative; i: inconclusive ; M: Multiplicat on fator to evaluate significant negative results. [b]Including simple rare firstains;[c]Considering mwh clones for simple stains and *mwh* for twin stains; SSS: Simple Small Stains; SLS: Simple Large Stains; TS: Twin Stains; TS: Total of Stains

DISCUSSION

The present research is the first to evaluate the cytotoxicity, acute toxicity, genotoxic, mutagenic and antimutagenic potential of *E. serratus* L. fruits in Brazil.

The results of the MTT assay in the study showed a moderated cytotoxicity from AEES. The assay is based on the activity of living cells converting the formazan crystals, and the increase or decrease of viable cell number shows the mitochondrial activity of the test substance [22]. This is the first time that the *E. serratus* species was tested by this assay. Other studies with other parts of the species such as the leaves [23] had their cytotoxicity evaluated by the brine shrimp assay, showing moderate cytotoxicity. Although moderate cytotoxic effects were observed in this study, other species from the *Elaeocarpaceae* family through the MTT assay towards human T4 lymphoblastoid (CEM-SS) and human cervical (HeLa) cancer cells did not present cytotoxic activity due its rich phenolic constitution [24].

The AEES did not show in the acute toxicity test any sign of toxic effects, evidenced by the absence of mortality in the evaluated animals and of behavioral and physiological changes. When there are no behavioral changes during the hipprocratic screening, no toxic signs, no macroscopic changes in the organs, no changes in feeding and water consumption, it means that the extract in the respective dose is not toxic [25,26]. The results of this study corroborate the findings of Geetha, Jayashree, Rajeswari [8], in a study that assessed the acute toxicity of the ethanolic extract of the *E. serratus* L. fruits at doses ranging from 1000 to 5000 mg/kg, where no mortality or any toxic effects were observed at all evaluated doses.

Genotoxicity assays are commonly used as cancer indicators since they can measure tumorigenesis events in their early or intermediate phase and are not considered measures of carcinogenesis [26]. To identify such agents and classify them as genotoxic or not, there is a range of in vitro and in vivo assays, such as comet assay, micronucleus test and *Drosophila melanogaster* that are commonly used in clinical practice and provide relevant and reliable information [27].

The comet assay allows the detection of direct injuries to the genetic material that is caused by genotoxic agents, whether synthetic or from medicinal plants, in addition to their repair mechanisms [18,27]. Treatment with the AEES showed no statistically significant differences compared to the Naive group ($p \leq 0.05$), confirming concomitantly with the findings of the micronucleus test, showing that administration of the AEES did not cause genotoxic effects. The micronucleus test allows the evaluation of substances that can cause clastogenic (that perform chromosome breakdown) and aneugenic (that induce abnormal chromosome segregation) damage [28]. By the micronucleus test, no statistically significant differences were observed in micronucleus frequency and PCE/NCE ratio with the AEES in all its fractions compared to the Naive group ($p \leq 0.05$), is only observed in the group treated with cyclophosphamide, as expected. The Smart assay can detect the mutagenic, deleterious and recombinant activity of natural, chemical, and synthetic compounds by using *Drosophila melanogaster* as a test organism to identify loss of heterozygosis [21]. Because D. melanogaster has 60% of orthologous genes to mammals, it is a reliable and rapid test and it is the primary test in associating genotoxic damage to substances after their biotransformation [29,30].

In the mutagenicity test by the SMART test performed in the study, the frequency of mutant stains in individuals resulting from ST and HB crossings, in groups treated with extracts and fractions of the AEES, does not differ statistically from the negative control ($p \leq 0.05$). Regarding the antimutagenicity test, there were no statistically significant differences in the frequency of mutant patches between groups treated with the AEES fractions and the negative control ($p \leq 0.05$), and all doses at the HB crossing and doses of 2.5 and 5.0 mg/ml at the ST crossing were able to reduce the effects of doxorubicin in somatic cells of D. melanogaster, presenting a percentage of reduction between 45.90% and 94.20%.

The mutations caused by doxorubicin are caused by the formation of adducts in DNA [31], induction of the formation of reactive oxygen species and single and double strand breaks in DNA [32]. Also, doxorubicin is an intercalating agent and can interact with topoisomerase II, causing permanent DNA lesions [33]. Cyclophosphamide, nitrogenous mustard, induces the formation of adducts that interfere with the crossing of guanine into DNA, where this constant alkylation can cause severe damage to genetic material and death of cells [34]. Antimutagenic agents act with distinct mechanisms of action: 1-desmutagenesis, when they alter the chemical or biochemical structure of mutagenic agents before damage occurs to the DNA molecule, and 2-bioantimutagenesis when they act on the suppression of mutation fixation after the DNA is damaged by the mutagenic agent [35]. The use of this class of compounds can be used to prevent the formation of tumors and some genetic diseases. In this context, the search for medicinal plants with anti-mutagenic and antioxidant potential is essential for cancer prevention, since they prevent the formation of these free radicals, reduce genetic damage, and make the action of chemotherapy more selective [36].

According to the findings of Freitas de Lima [12] the bioactive compounds of the pulp of *E. serratus* fruits are vitamin C, carotenoids, condensed tannins and, specially, flavonoids like quercetin and kaempferol were the most prevalent.

The flavonoids have a high antioxidant capacity [37] by the action of the hydroxyl group present in its constitution in removing free radicals caused by the chelation of metal ions [38], protecting the body from the toxic action of alkylating agents such as cyclophosphamide and antineoplastic drugs such as doxorubicin, which are considered highly genotoxic and mutagenic [39]. Given the antioxidant capacity of flavonoids, genotoxicity studies were conducted with isolated forms, such as quercetin and kaempferol, as in the study by Utesch [40], which assessed the genotoxicity of quercetin through the micronucleus test and the comet assay. The application of both tests resulted in no genotoxic effects being observed, corroborating the findings of the present study, where treatment with the AEES fractions did not lead to micronucleus formation and did not show cells damaged by the comet assay. The study by Sorbitrán, Ordaz-Telléz, Rodríguez-Arnaiz [41]. Proved that flavonoids such as quercetin and kaempferol are not genotoxic, attested by the SMART assay, corroborating the data in this study [42].

The results achieved by this research with *Elaeocarpus serratus* L. fruit extract demonstrate the importance of conducting toxicological tests to ensure safe consumption by the population, in addition to allowing new studies that can further explore the antimutagenic potential in the search for a new therapeutic agent for chemoprevention.

CONCLUSIONS

The AEES showed moderate cytotoxicity by the MTT assay. It presents low toxicity and the LD50 is higher than 2000 mg/kg. In this study, it did not show genotoxic potential in peripheral blood cells neither clastogenic nor aneugenic potential in cells derived from the bone marrow of rats. The extract is not mutagenic and presents antimutagenic activity, attested by the SMART test.

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