Evaluation of acute and subacute toxicity of the Brazilian *Jacaratia spinosa* (Aubl.) A. DC wild fruit extract in mice

Avaliação da toxicidade aguda e subaguda do extrato de frutos de *Jacaratia spinosa* (Aubl.) A. DC em camundongos

Evaluación de la toxicidad aguda y subaguda del extracto de frutos de *Jacaratia spinosa* (Aubl.) A. DC en ratones

Cellen Giacomelli Groth Luiz
ORCID: https://orcid.org/0000-0002-2030-8243
Federal University of Paraná, Brazil
E-mail: cellengroth@outlook.com
Gabriela Moraes Fortuna
ORCID: https://orcid.org/0000-0002-3329-9390
Federal University of Paraná, Brazil
E-mail: gabrielamoraesfortuna@gmail.com
Railson Henneberg
ORCID: https://orcid.org/0000-0003-3334-8567
Federal University of Paraná, Brazil
E-mail: railson@ufpr.br
Rubens Bertazolli Filho
ORCID: https://orcid.org/0000-0003-3784-4939
Federal University of Paraná, Brazil
E-mail: rbertazo@ufpr.br
Deise Prehs Montrucchio
ORCID: https://orcid.org/0000-0003-1440-7007
Federal University of Paraná, Brazil
E-mail: dpmontrucchio@ufpr.br
Elizabeth do Nascimento
ORCID: https://orcid.org/0000-0002-3618-2673
Federal University of Pernambuco, Brazil
E-mail: nlizbeth@gmail.com
Monica de Caldas Rosa dos Anjos
ORCID: https://orcid.org/0000-0002-8039-3398
Federal University of Paraná, Brazil
E-mail: monica.anjos@ufpr.br
Sila Mary Rodrigues Ferreira
ORCID: https://orcid.org/0000-0001-6118-6089
Federal University of Paraná, Brazil
E-mail: sila.ufpr@gmail.com

Abstract
Jacaratia spinosa (Aubl.) A. DC is an edible unconventional fruit rich in latex consumed by some Brazilian communities, and its toxicity has not been thoroughly tested. Thus, in vitro and/or in vivo tests are recommended to evaluate the safety of the fruit as food source. The present study evaluated acute and subacute toxicity of the aqueous extract of *Jaracatia spinosa* wild fruit (JE) administered orally in mice. At the end of subacute testing (28 days), the blood was evaluated for haematological and biochemical parameters and vital organs were submitted to histological analysis. No toxic effects were observed in the acute test. Subacute test, however, identified piloerection signals, increase in alanine transaminase and liver hypochromia. These results suggest that the subacute use of *Jaracatia spinosa* may produce signs of toxicity, thus suggesting that the consumption of fruits in high amounts or for a long time may produce adverse outcomes for health.
Keywords: Jaracatiá; Unconventional fruit; Toxicity; Hematologic evaluation; Biochemical analyses; Histological reviews.

Resumo
Jacaratia spinosa (Aubl.) A. DC produz um fruto comestível não convencional rico em látex, consumido em alguns locais do Brasil, cuja toxicidade ainda não foi testada. Dessa forma, testes in vitro ou in vivo são recomendados para

avaliar a segurança do fruto como fonte alimentar. O presente estudo avaliou a toxicidade aguda e subaguda de um extrato aquoso obtido dos frutos de *Jaracatia spinosa*, administrado em camundongos por via oral. Ao final do teste subagudo (28 dias), o sangue dos animais foi coletado para avaliação de parâmetros bioquímicos e hematológicos, e os órgãos vitais foram pesados e submetidos a análise histológica. Não foram observados efeitos tóxicos no teste agudo. Entretanto, no teste de toxicidade subaguda, foram identificados sinais de piloereção, aumento da enzima alanina transaminase e hipocromia nas células hepáticas. Esses resultados sugerem que o uso subagudo dos frutos de *Jaracatia spinosa* pode produzir sinais de toxicidade, sugerindo, portanto, que o consumo do fruto em grandes quantidades ou por longo período de tempo pode produzir resultados negativos à saúde.
Palavras-chave: Jaracatiá; Fruto não convencional; Toxicidade; Avaliação hematológica; Análise bioquímica; Histologia.

Resumen
Jacaratia spinosa (Aubl.) A. DC produce un fruto comestible no convencional rico en látex, consumido en algunas localidades de Brasil, cuya toxicidad aún no ha sido testada. Por lo tanto, se recomiendan pruebas in vitro o in vivo para evaluar la seguridad del fruto como fuente de alimento. El presente estudio evaluó la toxicidad aguda y subaguda de un extracto acuoso obtenido de los frutos de *Jaracatia spinosa*, administrado a ratones por la vía oral. Al final de la prueba subaguda (28 días), se extrajo la sangre de los animales para evaluación de parámetros bioquímicos y hematológicos, y los órganos vitales fueron pesados y sometidos a análisis histológicas. No se observaron efectos tóxicos em la prueba aguda. Sin embargo, en la prueba de toxicidad subaguda, fueron identificados signos de piloerección, aumento de la enzima alanina transaminasa y hipocromía en las células del hígado. Estos resultados sugieren que el uso subagudo de los frutos de *Jaracatia spinosa* puede producir signos de toxicidad, sugiriendo que el consumo del fruto em grandes cantidades o durante un largo período de tiempo puede producir resultados negativos para la salud.
Palabras clave: Jaracatiá; Fruto no convencional; Toxicidad; Evaluación hematológica; Análisis bioquímico; Histología.

1. Introduction

The native species *Jacaratia spinosa* (Aubl.) A. DC, belonging to the family Caricaceae, is distributed across South American countries (Tropicos, 2018, Flora de Nicaragua, 2018, The plant list, 2018). In Brazil, its cultivation predominates between the south of Bahia and the state of Rio Grande do Sul (Aguiar et al., 2012, Corteletti et al., 2015, Flora do Brasil 2020, 2018). According to Badillo (1971), *J. spinosa* has received several names, being known as *J. actinophylla*, *Papaya spinosa* and *Carica spinosa*, between the years 1775 and 1889, *J. dodecaphyllae* in 1902, and *J. costarricensis* in 1924.

Jaracatia spinosa has been referenced in a descriptive treatise about Brazil in 1587, prevailing in the Atlantic Forest biome, and also found in the Amazon rainforest and in the Cerrado (Souza, 1938; Ricardo et al., 2017, Flora do Brasil 2020, 2018). Its name derives from *yara-cati-á*, a word of indigenous origin meaning fragrant fruit (Souza, 1938) and it is natively known simply as jaracatiá.

The jaracatiá is considered an unconventional food plant, which bears fruit once a year, from January to March. Nowadays, some traditional communities prepare sweets from its fruit and stem for consumption. Jaracatiá´s sweets are regarded part of Brazil's cultural heritage (Kinupp & Lorenzi, 2014) and their consumption has gained importance as a food item of the usual diet. The Jaracatiá Festival, held in Southeast Brazil, has increased its inclusion in the diet by means of preparations such as ice creams, cakes, honey bread, butter cookies, compotes, bonbons, and cupcakes. The fruit is also used as juice in some southern Brazil communities (Bolson et al., 2015). It also has two antioxidant compounds, β-sitosterol and campesterol, which are closely linked to the antioxidant properties of food items (Abreu, 2015).

However, even in the maturity stage, the fruit presents a significant amount of latex, even higher than the unripe fruit of papaya. An ethnopharmacological review recognizes the anthelmintic properties of the jaracatiá latex, but warns that excessive consumption can lead to hyperthermia (Hoehne, 1946). In the edition of the book Formulary and Medical Guide (Chernoviz, 1874) the juice was indicated as vermifuge. The fruit is commonly used to make poultices for wounds, and its juice is recommended for stomach problems. However, excessive consumption of raw fruit has been reported as liable to cause abdominal discomfort and lip swelling (Hoehne, 1946). Some authors suggest that, prior to eating the raw fruit, longitudinal

incisions should be made in order to partially drain the latex, reporting that Guarani Indians consume the fruits after cooking or braising them (Martínez-Crovetto, 1968; Kinupp & Lorenzi, 2014). Previous non published studies using the fruit have resulted positive for haemolysis on sheep blood agar plate and positive for toxicity in brine shrimp lethality bioassay, thus suggesting the need of *in vivo* toxicological screening to determine the safety of the fruit used as a food source (Abreu, 2015).

Therefore, toxicological evidence indicates that any substance is potentially toxic, depending on the administered dose, the absorbed dose, and the exposure time (FDA, 2007; Khoo, 2010). Therefore, an unconventional plant must have its action and toxic level previously evaluated for verification of consumption-related safety. The popular and the traditional uses of this plant are not enough to validate it as safe (WHO, 2000; FDA, 2007). The acceptance and use of herbal plants have increased in recent years, in part because of the belief that these products are "safe" because they originate from natural sources. However, many of these agents have not been adequately tested, so little is known about their mode of action, adverse effects and contraindications, which compromises the safe use (Ekor, 2014; Balin et al., 2018). Regarding the toxicity assessment of traditional herbal medicines, Aydin et al. (2016) describe two types of screening: the single dose toxicity test (acute toxicity) as the first step in the toxicological analysis, and the repeated dose toxicity test, which aims to determine the *in vivo* effects of repeated daily exposure over periods of 28 days (subacute toxicity), 90 days (subchronic toxicity) or longer (chronic toxicity).

Despite few studies being reported in the literature, the consumption of jaracatiá fruit by the population has increased. Thus, this study aimed to evaluate the toxic effect of oral administration of the *Jaracatia spinosa* wild fruit crude extract, making use of international protocols for acute and subacute toxicity assays in mice.

2. Methodology

The present study is classified as an experimental research (Gil, 2002), which intend to verify possible toxic effects of jaracatiá fruit extract under controlled conditions. Toxicity tests were carried out according to standardized protocols from OECD (Organization for Economic Cooperation and Development), as described in the following sections.

2.1 Fruits

The *Jacaratia spinosa* (Aubl.) A. DC fruits were collected in Rolândia, Paraná State, south of Brazil (23°S14'48" 51°W24'43") in summer. Botanical identification was held at the herbarium of Municipal Botanical Museum of Curitiba, Paraná using the voucher specimen No. 379131. Authorization for access and shipment of samples of the genetic heritage component was given by the National Counsel of Technological and Scientific Development under No. 010004/2015-7.

2.2. Analysis and preparation of extracts

Fruits at the maturity stage, with at least 75% of their outer skin orange in colour, were selected, washed and sanitized. A hundred fruits were used in the physical analysis (shape, weight and length), which was performed in quintuplicate and with the whole fruit. The fruits had their stems removed and their skin, pulp and seeds were separated. The pulp was then grounded in a food processor Arno®, frozen (-18°C), and lyophilized (-20°C).

Chemical analysis of nature fruit, performed in triplicate, involved the determination of moisture in the natural fruit, assessment of other components (total proteins, lipids, total dietary fiber) in the freeze-dried fruit, and calculation of total carbohydrate by difference and pH (AOAC, 2005).

The extract obtained from jaracatiá fruits was used for *in vivo* assays with mice. It was prepared on a daily basis, by diluting the freeze-dried fruit pulp with distilled water and adding Tween 80 (5%) as emulsifying agent. Considering the intake

of 3, 6 and 12 fruits/day by a 65kg adult, the extract was prepared at concentrations of 1250 mg/kg, 2500 mg/kg and 5000 mg/kg for oral administration (Hor et al., 2012).

2.3 Animals

Male and female mice of the Swiss strain (*Mus musculus*) were used at eight weeks of age, weighing between 25 and 35g. The animals were placed in opaque polypropylene cages, divided into groups of three mice for the acute toxicity test, and five mice for the subacute toxicity study. The cages were lined with wood shavings and kept in a temperature-controlled room at $22 \pm 2°C$, under 12-hour light/dark cycle, with *ad libitum* access to food (commercial feed AIN-93) and drinking water. The animals were acclimatized to the laboratory conditions for seven days prior to the beginning of experiments (Damy et al., 2010). In both tests, behavioural and physiological signals were evaluated, such as changes in the skin, fur, eyes, mucous membranes, breathing, muscle tone, motor activity, convulsions, salivation, diarrhea, lethargy, body temperature and weight. Four levels of piloerection were distinguished, namely: lack of signs, rare signs, clear signs, clear and continuous signs (Malone and Robichaud, 1983; OECD, 2001; OECD, 2008).

The experimental protocol was approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Paraná, under No. 869. The whole experiment was performed in compliance with the principles established by the Normative Resolutions of the National Council for the Control of Animal Experimentation (CONCEA), according to the provisions of the current legislation (Brazil, 2008) and the ARRIVE guidelines (Animal Research: Reporting of *In Vivo* Experiments) (Kilkenny et al., 2013).

2.3.1 Acute toxicity test

The acute toxicity test was conducted following the OECD guideline 423 (2001), using only female mice (n=6 per dose), for these are more sensitive to toxic effects. The technique consisted in the administration of the jaracatiá extract (JE) at a single dose of 2000 mg/kg in three mice, in order to verify the capacity to cause death to 50% of the animals within a 14-day period. As no death was observed, an identical dose was administered to three other mice, which were observed for 14 days. As no death occurred yet, a dose of 5000 mg/kg could then be administered, according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (OECD, 2001). During all the experiment, the animals were given *ad libitum* access to food and water.

2.3.2 Subacute toxicity study

The subacute toxicity study was conducted with 40 animals divided into four groups, with five mice of each sex per group, for 28 days, following the OECD 407 (2008). The animals were divided as following: control group, which received only the vehicle (distilled water and 5% Tween 80), and groups 1250, 2500 and 5000, that received the jaracatiá extract (JE) at doses of 1250, 2500 and 5000 mg/kg/day, respectively. The animals were evaluated daily for behavioural and physiological signals, as described on item 2.3. On the 28th day, allowing a 60-minute interval after the administration of the last dose, the animals were submitted to the open field locomotor activity test, which consisted in placing the animal inside a wooden box (50x50cm) with its floor divided into 16 squares of equal size. The number of squares crossed with all four paws within a six-minute period was then counted (OECD, 2001; Montrucchio, 2012). On the 29th day, the animals were fasted for 12 hours with free access to drinking water, prior to the collection of 1.0 mL of blood, of which 0.2 mL was packed in a tube with EDTA, and 0.8 mL in a dry tube. For this, the animals were anesthetized through intraperitoneal injection of a ketamine (50 mg/kg) and xylazine (10 mg/kg) combination. Whole blood was used for automated hematologic analysis (Horiba Micros ABX®) according to standard procedures. The reading of haematological slides was performed using optical microscopy.

Biochemical analyses were performed in serum by automation (LabMax 400® Labtest®) after centrifugation of coagulated blood at 3500 rpm for 10 minutes. The analyses addressed the liver functions (aspartate aminotransferase - AST, alanine aminotransferase - ALT, alkaline phosphatase), kidney functions (urea, creatinine, uric acid), and other biochemical parameters (glucose and total cholesterol) using specific Labtest® kits.

After euthanasia, vital organs such as heart, lungs, liver, spleen, kidneys, stomach and intestines were removed, weighed and examined macroscopically. The relative organ weight was calculated based on the equation: *(organ weight/body weight) x100*. Following that, the organs were cut and fixed in formalin solution (10%), dehydrated, diaphanized, embedded in paraffin, and cut into 5μm-thick sections with Leica® Rm 2145 microtome. The cuts were fixed into glass slides, stained with Harris hematoxylin solution, photographed in light microscope Zeiss (Axio Lab A1) coupled to AxioCam ERc5s camera, and processed in ZEN Digital Imaging program. All microscopic analyses were conducted in a blinded fashion for the groups. Tissue samples were structurally evaluated by observing damage, congestion or bleeding, necrosis, fibrosis, leucocyte infiltration, fatty degeneration, and accumulation of bile pigments.

2.4 Statistics

Statistical analyses were performed using the software Prisma® (Graphpad Prism 5.0 Software Inc. La Jolla, CA, USA). For organ weight evaluation, hematologic and biochemical analyses, one-way analysis of variance (Anova) was performed, followed by Tukey's test. For assessment of weight gain, the two-way Anova was used followed by Bonferroni´s test. A 5% significance level was considered for statistical purposes. The results are expressed as mean and standard deviation.

3. Results

3.1 Characterization of fruits

Jaracatiá fruits are indehiscent, fleshy, oval or pear-shaped, berrylike, with seeds and the physical appearance similar to the papaya. The fruits have an average weight of 58.263 g (± 15.483) and a length of 6.94 cm (± 0.91). The chemical characterization of *in natura* fruits showed 85.43% (± 0.641) moisture, 2.35% (± 0.061) total proteins, 0.17% (± 0.001) lipids, 11.13% (± 0.652) carbohydrate, 4.47% (± 0.021) dietary fiber and acidity of 4.46.

3.2 Acute toxicity test

The administration of JE produced no lethal effects at doses of 2000 mg/kg and 5000 mg/kg. However, after administration the animals showed contractions in the abdominal region, evidencing signs of abdominal cramps, and performed the act of cleaning themselves up. Piloerection and softened stools were persistent until the 3rd day in all animals of both groups. In the group that received 2000 mg/kg, 50% of animals decreased ambulation during the first 2 hours, and 50% of animals slept. No toxic effect was detected after the 3rd day.

3.3 Subacute toxicity study

3.3.1 Clinical signs

During the 28 days of treatment with JE, no death was observed. A decrease in ambulation, cleaning behaviour and signs of abdominal cramps were observed in 100% of treated animals. However, the open field motor activity test showed no statistical difference between treated and control groups. Regarding the evacuation, the treated animals showed an increase in defecation frequency compared to the control group. Such increase was gradual, according to the dose used, for both males and females. In the group 1250, the number of faecal boli compared to the control group increased by 77.4% for females and 90.6% among males. At the dose of 2500 mg/kg, the increase reached 109.6% and 139.6% for females and males, respectively.
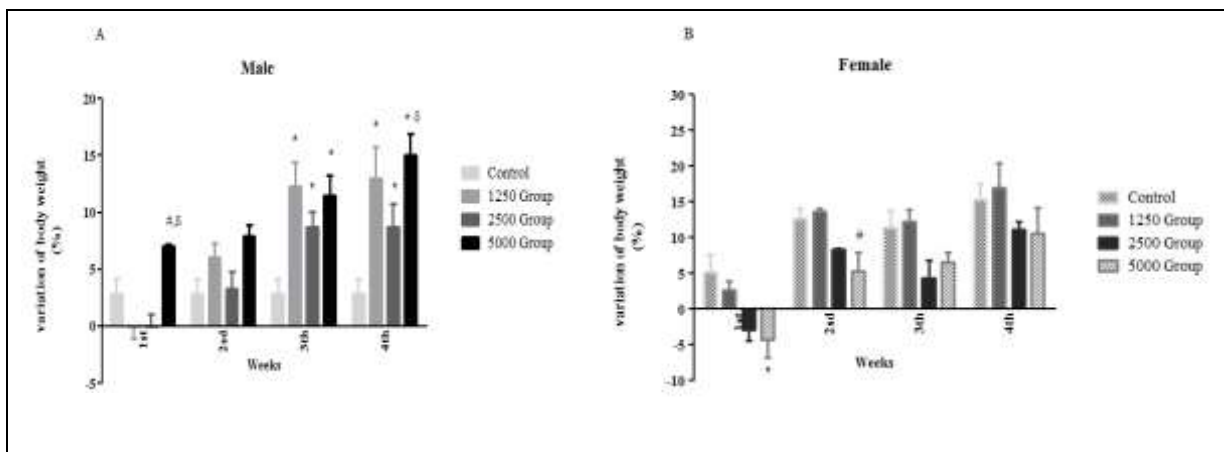
At the dose 5000 mg/kg, the number of faecal boli increased by 151.6% in females and 254.7% in males. Besides the increase in the number, faecal boli were around 30% larger in diameter, compared to control group. Softening of feces was also observed in the treated groups.

There were no changes regarding temperature, cyanosis signs, lachrymation, hypnosis or anesthesia. Regarding piloerection, a dose-response effect was observed. In the control group, 17% of females and 8% of males showed rare signs of piloerection, while others in the group had no change in the fur. In the group 1250, 8% of males and 33% of females showed clear signs of piloerection, while 92% of males and 67% of females presented clear and continuous signs of piloerection. For the groups 2500 and 5000, both genders showed clear and continuous signs of piloerection in all animals.

3.3.2 Body mass gain

The treated animals showed significant statistical differences in mass gain in comparison to the control group at week 1, a period when some of these animals showed a weight loss. After the first week, the greatest differences in body mass occurred in males, which gained more weight than the control group (Figure 1). In the third and fourth weeks, the weight increase was higher than control group in all male JE treated groups (Figure 1 A), while among females, only group 5000 in the second week had weight increase statistically different from control group (Figure 1 B).

Figure 1. Effect of jaracatiá extract (JE) on body weight gain of male (A) and female (B) mice in the subacute toxicity study.
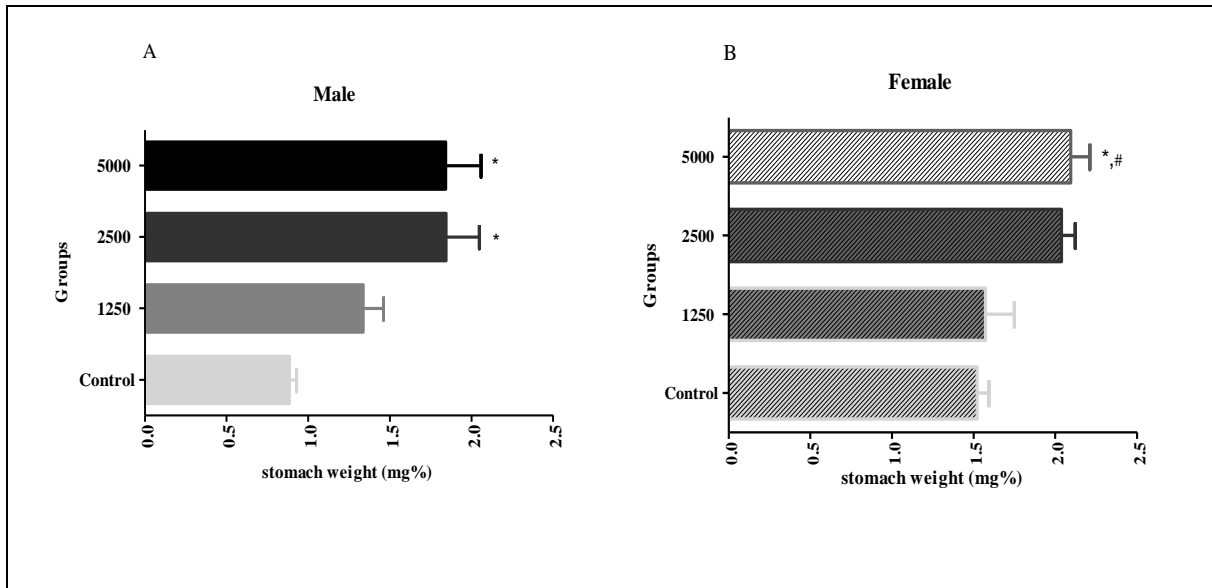

Note: Control group and JE treated groups at doses of 1250, 2500 and 5000 mg/kg. Values are expressed in percentage compared to the first day of treatment and the bars indicate the standard error of mean. Significance expressed as: *p<0,05 *vs* Control; #p<0,05 *vs* 1250; δp<0,05 *vs* 2500. Two way ANOVA followed by Bonferroni test.
Source: The authors

3.3.3 Relative organs weight

The oral administration of JE caused a significant increase in stomach weight in the groups 2500 and 5000, in both males (p=0.0018) and females (p=0.0083), compared to the control group (Figure 2). Macroscopic examination of mice stomachs in groups 1250, 2500 and 5000 revealed hyperemia with gastric wall thickening in 100% of animals.

Figure 2. Effect of jaracatiá extract (JE) on relative organ weight (stomach weight/body weight %) of male (A) and female (B) mice in the subacute toxicity study.

Note: Control group and JE treated groups at doses of 1250, 2500 and 5000 mg/kg. Values are expressed in percentage and significance is expressed as: *$p<0,05$ *vs* Control; #$p<0,05$ *vs* 1250. One way ANOVA followed by Tukey test.
Source: The authors

Relative weight of liver, spleen, kidneys and lungs are showed in Table 1. Differences from control group were observed only in the relative weight of kidneys: an increase in the weight of renal mass was observed in females at the dose of 5000 mg/kg ($p=0.0314$); and a decrease in renal mass of males occurred in the group 2500 ($p=0.048$).

Table 1. Effects of *Jacaratia spinosa* (Aubl.) A. DC extract on relative organ weight (organ weight/body weight%) in male and female Swiss mice treated orally for 28 consecutive days.
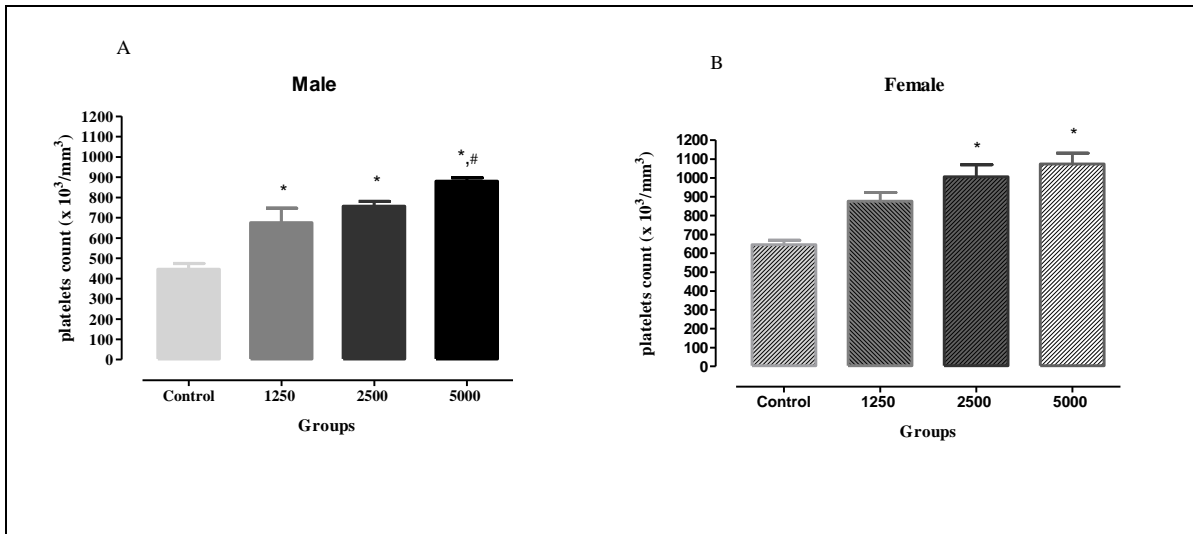
Organs (weight/body weight%)	Female (n=5)	Dose (mg/kg/day)				Male (n=5)	Dose (mg/kg/day)			
	Control	1250	2500	5000	p-value	Control	1250	2500	5000	p-value
Liver	38.49 [a] ± 4.09	44.04 [a] ± 2.94	43.17 [a] ± 5.60	42.04 [a] ± 4.83	0.2092	41.57 [a] ± 2.72	38.56 [a] ± 1.82	38.60 [a] ± 5.23	38.72 [a] ± 1.35	0.7175
Spleen	4.14 [a] ± 0.30	3.67 [a] ± 0.25	3.82 [a] ± 0.84	3.44 [a] ± 0.67	0.2062	2.99 [a] ± 0.37	2.78 [a] ± 0.38	3.10 [a] ± 1.07	3.17 [a] ± 0.49	0.8072
Kidneys	9.88 [a] ± 0.67	11.26 [ab] ± 1.34	12.16 [ab] ± 2.09	12.22 [b] ± 1.33	0.0314	14.34 [a] ± 1.02	13.87 [a] ± 1.74	10.93 [b] ± 2.16	15.59 [b] ± 0.17	0.0483
Lungs	5.33 [a] ± 0.65	5.39 [a] ± 0.53	6.15 [a] ± 0.52	5.73 [a] ± 0.26	0.0557	5.70 [a] ± 0.34	5.79 [a] ± 1.12	5.24 [a] ± 0.72	5.34 [a] ± 0.53	0.6832

Note: Values expressed as mean ± SD. Means with the same letter in a row do not differ ($p<0.05$). One way ANOVA followed by Tukey test.
Source: The authors

3.3.4 Hematologic evaluation

After 28 consecutive days of oral treatment with JE, the results showed in Figure 3 demonstrate a significant increase in platelet count for males ($p<0.0001$) and females ($p=0.0003$) of groups 1250, 2500, and 5000, compared to the control group. It was also observed an increase in segmented neutrophils in males ($p=0.0026$) and females ($p=0.0056$) of groups 2500 and 5000 when compared to control group. No differences were observed for the other hematological parameters.

Figure 3. Effect of jaracatiá extract (JE) on platelets count of male (A) and female (B) mice in the subacute toxicity study.

Note: Control group and JE treated groups at doses of 1250, 2500 and 5000 mg/kg. Values are expressed as mean ± SEM and significance is expressed as: *$p<0,05$ *vs* Control; #$p<0,05$ *vs* 1250. One way ANOVA followed by Tukey test.
Source: The authors

3.3.5 Biochemical analysis

The results obtained for biochemical parameters are demonstrated on Table 2. A significant increase in alanine transaminase (ALT) ($p=0.0048$) was observed in males of groups 2500 and 5000, when compared to the control group. Alkaline phosphatase was statistically different from control ($p<0.0001$) in males of all treated groups, with the greatest increase found in the group 5000. In females, the increase in serum alkaline phosphatase levels was significant ($p<0.0001$) in groups 2500 and 5000, compared to the control group, but no differences were observed in the intra-group evaluation. Regarding aspartate aminotransferase, urea, creatinine, uric acid, glucose and total cholesterol, no significant changes were found for any of the administered extract doses.

Table 2. Effects of *Jacaratia spinosa* (Aubl.) A. DC extract on biochemical parameters in male and female Swiss mice treated orally for 28 consecutive days.

Parameters	Female (n=5)		Dose (mg/kg/day)			Male (n=5)		Dose (mg/kg/day)		
	Control	1250	2500	5000	p-value	Control	1250	2500	5000	p-value
ALT (U/L)	42.40 [a] ± 20.12	39.60 [a] ± 11.84	38.20 [a] ± 21.51	47.50 [a] ± 20.99	0.8510	16.20 [a] ± 4.44	16.20 [a] ± 1.92	26.00 [b] ± 5.96	29.00 [b] ± 2.16	0.0048
AST (U/L)	102.20 [a] ± 39.30	94.40 [a] ± 19.44	108.60 [a] ± 49.66	92.33 [a] ± 10.54	0.4867	63.40 [a] ± 13.65	48.00 [a] ± 4.00	64.80 [a] ± 19.50	44.75 [a] ± 6.24	0.0618
Urea (mg/dL)	42.40 [a] ± 7.50	43.20 [a] ± 11.97	36.40 [a] ± 4.83	48.67 [a] ± 9.48	0.1775	49.40 [a] ± 9.42	46.20 [a] ± 6.14	51.80 [a] ± 6.14	43.50 [a] ± 6.61	0.3659
Creatinine (mg/dL)	0.43 [a] ± 0.15	0.36 [a] ± 0.17	0.43 [a] ± 0.11	0.36 [a] ± 0.10	0.7013	0.40 [a] ± 0.12	0.37 [a] ± 0.02	0.40 [a] ± 0.09	0.31 [a] ± 0.04	0.3715
Glucose (mg/dL)	107.60 [a] ± 10.74	145.80 [a] ± 39.21	152.00 [a] ± 46.23	122.17 [a] ± 38.87	0.2038	124.00 [a] ± 22.06	118.20 [a] ± 28.99	109.20 [a] ± 27.37	130.25 [a] ± 31.08	0.6940
Uric Acid (mg/dL)	3.89 [a] ± 0.43	3.91 [a] ± 0.57	4.44 [a] ± 0.41	3.81 [a] ± 0.62	0.3606	1.74 [a] ± 0.31	1.72 [a] ± 0.41	1.98 [a] ± 0.40	2.20 [a] ± 0.28	0.2000
Cholesterol (mg/dL)	115.60 [a] ± 4.88	121.20 [a] ± 12.79	131.40 [a] ± 7.89	127.83 [a] ± 8.61	0.3134	123.00 [a] ± 2.25	142.20 [a] ± 16.99	147.20 [a] ± 12.34	146.00 [a] ± 12.54	0.0529
Alkaline Phosphatase (U/L)	93.20 [a] ± 14.51	120.40 [a] ± 42.90	173.40 [b] ± 36.53	197.33 [b] ± 21.12	<0.0001	89.40 [a] ± 4.48	126.60 [b] ± 24.39	150.60 [b] ± 10.43	167.50 [c] ± 12.69	<0.0001

Note: Values expressed as mean ± SD. Means with the same letter in a row do not differ (p<0.05). One way ANOVA followed by Tukey test.
Source: The authors.

3.3.6 Histological assays

The histological changes in organs of males and females in the different groups are described in Table 3 and showed in Figure 4. The results showed that jaracatiá extract led to greater histological changes in the stomach, liver and spleen. Changes in the gastric mucosa occurred in 60 - 80% of animals treated with all doses of extract. Moreover, some animals in groups 1250 and 2500 showed changes in the gastric cardia, while in male animals of group 5000, leukocyte infiltration was also observed. In the liver, vascular congestion was observed in around 80% of animals treated with all doses of extract. Hepatocyte vacuolation was also common in treated groups, as well as hepatic subcapsular ischemia, visually characterized by hypochromia, which occurred in both genders of all treated groups, with marked increase in the frequency among females of groups 2500 and 5000. In spleen, most frequent alteration was vascular congestion and increase in megakaryocytes. Mild vascular congestion occurred in 20% of animals in control group, with preservation of the white pulp and normal cellularity in the red pulp. In all treatment groups, the congestion frequency was higher in females and similar to the control group in males. An increase in the number of megakaryocytes was also observed in the treated animals, markedly in groups 2500 and 5000. In the group 1250, disorganized white pulp was observed in both genders, and increased red pulp was present in females. Hypercellular white pulp was also observed in females of groups 2500 and 5000. Mild vascular congestion was also observed in kidneys of treated animals.

Table 3. Histological alterations in vital organs of male and female Swiss mice treated orally with *Jacaratia spinosa* (Aubl.) A. DC fruit extract for 28 consecutive days.

Parameters	Control Group		Group 1250		Group 2500		Group 5000	
	Female	Male	Female	Male	Female	Male	Female	Male
Stomach								
Vascular congestion	-	-	-	-	-	-	1+ 40%	
Leukocyte infiltration	-	-	-	-	-	-	-	1+ 20%
Affected gastric mucosa	20%	20%	80%	80%	80%	80%	80%	60%
Affected gastric cardia	-	-	20%	-	-	20%	-	-
Liver								
Vascular congestion	1+ 20% 2+ 20%	1+ 40%	1+ 80%	1+ 80%	1+ 40%	1+ 100%	1+ 80%	1+ 60% 2+ 20%
Hepatocyte vacuolation	1+ 20%	-	1+ 80%		1+ 80%	1+ 20%	1+ 80%	1+ 20%
Poorly defined hepatocyte	-	-	80%	20%	80%	20%	20%	-
Poorly defined sinusoids	-	-	-	20%	-	-	-	20%
Subcapsular Hypochromia	-	-	20%	20%	100%	40%	60%	20%
Jejunum								
Well-developed GALT	-	-	-	-	-	-	20%	-
Spleen								
Vascular congestion	1+ 20%	1+ 20%	1+ 40% 2+ 60%	1+ 20%	1+ 80%	1+ 20%	1+ 40%	1+ 20%
Increase in megakaryocytes	-	-	1+ 40% 2+ 20%	1+ 40% 2+ 20%	1+ 80%	1+ 80%	2+ 80%	1+ 20%
Disorganized white pulp			40%	40%		20%		
Increase in the red pulp	-	-	40%	-	-	-	-	-
Hypercellular white pulp	-	-	-	-	60%	-	100%	-
Lung								
Vascular congestion	1+ 20%	-	1+ 80%	1+ 20%	1+ 60%	1+ 80%	1+ 80%	1+ 20% 2+ 20%
Leukocyte infiltration	1+ 40%	-	1+ 80%	1+ 40%	1+ 40%	1+ 60% 2+ 20%	1+ 40% 2+ 60%	1+ 40%
Alveolar thickening	20%	20%	80%	80%	80%	80%	100%	60%
Granulomas	-	-	-	20%	-	-	20%	-
Bronchial secretion	-	-	-	-	-	20%	-	-
Kidney								
Vascular congestion	1+ 20%	-	1+ 100%	1+ 60%	1+ 60%	1+ 80%	1+ 100%	1+ 40%
Leukocyte infiltration	-	-	1+ 20%	1+ 20%	-	-	-	-
Tubule with increased acidophilia	-	-	-	-	-	-	80%	-

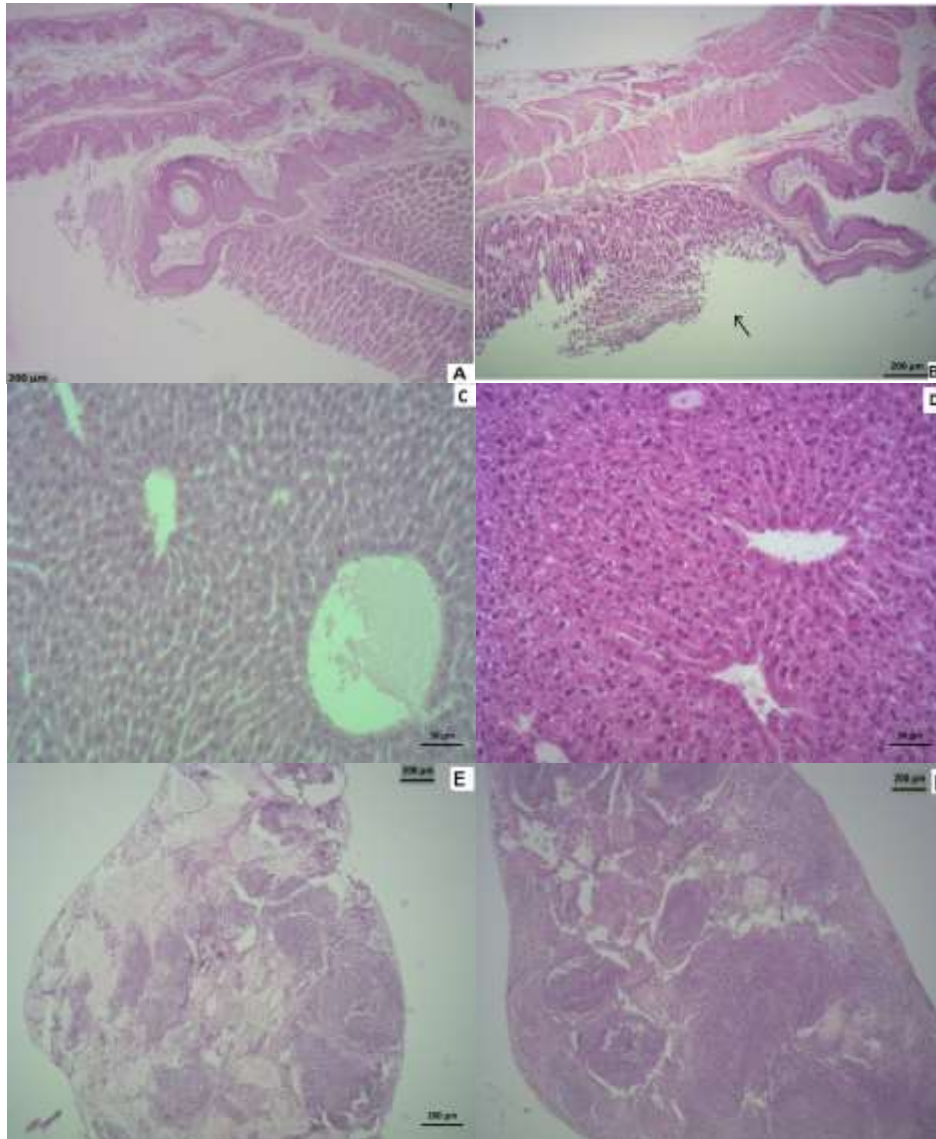Note: Frequency of histopathologic changes events. Five animals per gender per group.
Scores: - Absent; 1+ Mild; 2+ Moderate; 3+ Severe.
GALT: gut-associated lymphoid tissue
Source: The authors

The main alterations described in Table 3 may be visualized in the Figure 4. Changes in the gastric mucosa of animals that received the extract (Figure 4 B) were more frequent than in the control group (Figure 4 A). In the liver, vascular congestion and poorly-defined vacuolated hepatocytes were observed in all groups that received the jaracatiá extract (Figure 4 D) when compared to control group (Figure 4 C). Histological analysis of the spleen showed, in the control group (Figure 4 E), preservation of the white pulp and normal cellularity in the red pulp. In treated groups (Figure 4 F), the congestion frequency was higher in females, with an increase in the number of megakaryocytes. Disorganized white pulp was observed in both genders of groups 1250 and increased red pulp was present in females.

Figure 4. Histopathological analysis of stomach, liver and spleen of Swiss mice after 28 consecutive days of treatment with *Jacaratia spinosa* (Aubl.) A. DC fruit extract.

Note: Histopathological analysis (HE x 100) of stomach (A and B), liver (C and D) and spleen (E and F). Control group (A, C, E) and JE (5000 mg/kg/day) treated group.
Source: The authors.

4. Discussion

Regarding acute toxicity, the criteria adopted by the OECD (2001) indicate that when the dose of 5000mg/kg results in death of up to one animal, the product is classified in GHS 5. In the present study, no animal death occurred at this dose, thus the LD_{50} for the *Jaracatia spinosa* raw fruits was above 5000 mg/kg, featuring a relatively low acute toxicity (OECD, 2001; UNECE, 2011).

Among the animals subjected to the subacute toxicity test, it was observed alterations in faecal boli of all animals. It may be suggested that the increased frequency of defecation and change in feces consistency are due to the chemical composition of the fruit, particularly the presence of the latex, as well as the high content of dietary fiber. Holstege (2005) reports that plants of the gender *Solanum*, which are milky, as is the case of jaracatiá, can produce diarrhea in humans when in chronic exposure. The proposed mechanisms involve the anthraquinones present in the latex, which affect the chloride

channels in the cell membrane, blocking the absorption of electrolytes in the mucosa of the large intestine and leading to increased local pressure and intestinal peristalsis. In line with this, Almança et al. (2011) described diarrhea in rats receiving 1.4 g/kg of *Solanum cernuum* hydroalcoholic extract in subchronic testing. The authors also report that the hydroalcoholic extract of *Solanum cernuum* at doses of 2.4, 8, 12, 16, 20, and 25 g/kg in rats evidenced intense signals of piloerection (Almança et al., 2011), a feature that was also observed in the mice treated with JE in the present study.

In normal conditions, it is expected that animals gain weight along the weeks. In the present study some treated animals lost weight during the first week, and then had weight increases in the following weeks. Among treated groups, male animals had more significative weight increases. The major concern regarding the body mass evaluation would be the low weight gain or even weight losses, that could be, by itself, indicative of toxicity (OECD, 2008). In this case, as weight losses occurred only in the first week, it may be suggested that during this period occurred an adaptation of the organism to the use of JE.

When vital organs were isolated for evaluation, it was observed that the higher doses of JE caused increases in stomach relative weight, as well as thickening of gastric wall and hyperemia, signs that could be suggestive of chemical injury (Odze and Goldblu, 2015). In a study by Boudreau et al. (2013), there was stomach hyperplasia in mice that consumed aloe vera extract (*Aloe barbadensis* Miller) in its unpurified (nondecolorized) form, i.e., the crude extract with a high content of latex, as which jaracatiá extract. Among the other evaluated organs, only kidneys had significative changes to the control, with relative weight increases at the higher doses. Luyong et al. (2004) reported increased kidney weight caused by inflammatory infiltrate in animals that had consumed anthraquinones present in rhubarb. It may be suggested that this same effect occurred in animals treated with high doses of JE due to the presence of anthraquinones in jaracatiá latex. A previous study assessing the toxicity of *Aloe vera* reported a reduction in renal mass in females that were given 4% dose over a year of use. Such differences in kidney weights were specifically assigned to the hydration of the animals, given its laxative effects (Matsuda et al., 2008).

Regarding hematologic parameters, although an increase in platelet count were found in all JE treated groups, these values correspond to the benchmarks established by Restell et al. (2014). However, since the platelets are inflammatory cells that play an important role in body defence, platelet disorders may occur primarily in response to acute hemorrhage or reactive thrombocytosis secondary to inflammation. Additionally, chronic diseases, disorders in the digestive tract, endocrine imbalance, and tissue damage can be causes of thrombocytosis (Bleeker & Hogan, 2011; Rose et al, 2012). The presented results corroborate previous studies, such as the one by Voss and Brennecke (1991), who observed thrombocytosis in a subchronic test over 30 days in rats treated with *Cassia obtusifolia* (0.50%). The authors attributed the toxicity observed to the anthraquinone present in the latex, and it may be suggested a similar mechanism for the extract tested in the present study. Similarly, in a subchronic trial with crude extract of *Carica papaya* leaves, the platelet count increased significantly in the treated group (Dharmarathna et al., 2013). Jaracatiá extract also caused an increase in segmented neutrophils at doses 2500 and 5000 mg/kg. As these cells are the main agents of acute inflammation and immunologic response, these results suggest some kind of injury in animals treated with higher doses of extract (Kolaczkowska & Kubes, 2013). The other hematological parameters were not affected by JE. Similar to the current study, Hor et al. (2011) performed a subchronic test in mice with an exotic fruit, using methanol extract of pitaya (dragon fruit) in the same dosages (1250, 2500, and 5000 mg/kg), which also did not produce any significant differences in the hematologic evaluations. In females of group 5000, there was an increase in the erythrocyte count, compared to the control group (p=0.0417). The same was not observed in males.

Regarding biochemical parameters, the increase of alanine transaminase (ALT) and alkaline phosphatase levels in groups treated with the higher doses of JE may suggest liver damages such as necrosis or changes in blood capillary membrane permeability (Silva et al., 2015). Accordingly, Almança et al. (2011) observed a significant increase in ALT and AST

(aspartate transaminase) activities in mice that received the hydroalcoholic extract of *Solanum cernuum* at lower (0.1 g/kg) to higher doses (1.4 g/kg), corroborating the present findings.

As described in the results section, histological alterations in stomach, liver and spleen were observed in treated animals. These findings are in accordance to the other evaluated parameters of toxicity, such as the increase in relative weight of stomach and the alterations in hepatic enzymes content. Similarly to the present results, Boudreau et al. (2013) observed that the consumption of the unpurified crude extract of *Aloe vera* leaves in drinking water by rats and mice for 13 weeks [0%, 1%, 2% or 3% (w/w)] and 2 years [0%, 0.5%, 1%, or 1.5% (w/w)], increased the incidence and severity of goblet cell hyperplasia in the large intestine of animals of both genders, compared to the control group. Such results are consistent with other studies involving *Aloe vera* unpurified preparations, in which high amounts of anthraquinone were found (Matsuda et al., 2008; Yokohira et al., 2009). The evidences in the literature suggesting carcinogenic effects related to anthraquinones (Boudreau & Beland, 2006), led the FDA to ban the use of drugs containing this substance in the unpurified form.

The histological alterations found in liver of treated animals are similar to other publications. Preliminary test using the milky plant *Senna occidentalis* at 4% for 28 days evidenced liver hypochromia in treated rats (Barbosa-Ferreira et al., 2005). Holstege (2005) showed that, in plants belonging to the genus *Solanum*, inhibition of liver microsomal enzymes cause hemolysis in acute or short-term exposure. Anthraquinones have been shown to be hepatoprotective at low dosages (therapeutic doses), but have shown hepatotoxic effects with use of increasing doses (Wang et al., 2011). Therefore, given the histological findings of this study and the presence of anthraquinones in the jaracatiá latex, we hypothesize that the observed effects are partially due to the presence of these compounds.

The presence of megakaryocytes in the spleen may be related to compensatory mechanisms after thrombocytopenia (Machlus and Italiano, 2013). The presented results showed a thrombocytosis status (Table 3), that might be reactive, secondary to thrombocytopenia. Barbosa-Ferreira et al (2005) also reported reduction in spleen white pulp of adult rats treated with low concentrations (up to 1%) of *Senna occidentalis* seeds, thus confirming its toxicity.

5. Conclusion

Toxic effects were not observed in the acute test. Subacute testing for 28 days, however, demonstrate changes in hematologic parameters with increase in the platelet count and neutrophils, both suggestive of inflammatory status. Biochemical alterations in ALT and alkaline phosphatase levels were also observed when compared to control group, although still within the reference levels for the species. It was also observed an increase in the relative weight of the stomach, as well as histological changes in stomach, liver and spleen, aligned with the biochemical findings. Such results indicate that the consumption of these fruits in high amounts or for a long time may produce signs of toxicity.

As the dosages and frequency of consumption may not represent the reality of communities, further studies are recommended to evaluate the effects of the long-term consumption of the *Jaracatia spinosa* fruits at low doses. Such studies may include subchronic (90 days) or chronic (longer than 90 days) assays of toxicity, as well as exploratory approaches directly with the communities that consume jaracatiá fruits regularly.

Acknowledgments

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