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Sacha Inchi (*Plukenetia volubilis* L.) powder: acute toxicity, 90 days oral toxicity study and micronucleus assay in rodents

[Sacha Inchi (*Plukenetia volubilis* L.): toxicidad aguda, toxicidad oral durante 90 días y ensayo de micronúcleos en roedores]

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Abstract

Context: Sacha Inchi has been consumed for years by indigenous peoples. Meanwhile, its toxicological potential has not been sufficiently studied.

Aims: To assess the acute, sub-chronic toxicity and genotoxicity evaluation of Sacha Inchi powder obtained from *Plukenetia volubilis* L.

Methods: A dose of 2000 mg/kg was orally administered to rats and mice and toxicity symptoms for 14 days were observed. In repeated dose study, the product was orally administered to Sprague Dawley rats of both sexes. Animals received 50, 250 and 500 mg/kg/day of the product for 90 days. At the end, animals were sacrificed and samples were done for hematological and biochemical analysis, organ weighs and histopathological examination. Genotoxicity potential of Sacha Inchi powder was evaluated through micronucleus test in mice. Negative controls received the vehicle (carboxymethyl cellulose, 0.5%) used.

Results: No morbidity or mortality at 2000 mg/kg of the product were found. Sacha Inchi powder oral administration during 90 days to rats did not lead to death, body weight gain, food consumption, or adverse events. No significant changes on hematological or biochemical parameters, organ weights or histopathological findings were observed. Induction of micronucleus formation attributable to product was not found in mice.

Conclusions: No toxicity effects after oral acute exposure of Sacha Inchi power to rats and mice were observed. Neither toxicity attributable to oral doses of the product up to 500 mg/kg during 90 days to rats were found. Results suggested Sacha Inchi powder does not have genotoxicity potential under our experimental conditions.

Keywords: acute toxicity; micronucleus assay; Sacha Inchi; subchronic toxicity; toxicological studies.

Resumen

Contexto: Sacha Inchi ha sido consumido por años por poblaciones indígenas. Sin embargo, su potencial toxicológico no ha sido suficientemente estudiado.

Objetivos: Evaluar la toxicidad aguda, subcrónica y el potencial genotóxico del polvo de Sacha Inchi obtenido de *Pluketenia volubilis* L.

Métodos: Fue administrada una dosis oral 2000 mg/kg a ratas y ratones, y la aparición de síntomas tóxicos durante 14 días fueron observados. El polvo de Sacha inchi fue administrado por via oral a ratas de ambos sexos (50, 250 y 500 mg/kg/día) durante 90 días. Al finalizar, los animales fueron sacrificados y se tomaron muestras para análisis hematológicos y bioquímicos, peso de órganos y examen histopatológico. El potencial genotóxico del producto fue evaluado a través del ensayo de micronúcleos en ratón. Los controles recibieron el vehículo utilizado (carboximetilcelulosa 0,5%).

Resultados: No se detectó mortalidad, ni morbilidad tras la exposición a dosis orales de 2000 mg/kg. El polvo de Sacha Inchi no indujo muertes, cambios en la ganancia de peso, consumo de alimentos, ni eventos adversos. No se detectaron cambios significativos en los parámetros hematológicos y bioquímicos evaluados, peso de los órganos y hallazgos histopatológicos detectados. El producto no indujo la formación de micronúcleos en ratones.

Conclusiones: El polvo de Sacha Inchi no produjo efectos tóxicos agudos en ratas ni en ratones. La administración de dosis orales durante 90 días hasta 500 mg/kg a ratas tampoco conllevó a la aparición de toxicidad atribuible al tratamiento. El producto no es genotóxico en las condiciones de ensayo.

Palabras Clave: ensayo micronúcleos; ensayo toxicológico subcrónico; Sacha Inchi; toxicidad aguda.

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INTRODUCTION

The use of plant species as traditional medicines or nutraceuticals provides a good substitute in healthcare services for rural communities of the developing countries. It has been estimated that around 80% of the population in developing countries depends on traditional medicines for primary health care system. Medicinal plants are the indigenous heritage of global importance. In this way, today research on traditional uses of plant species has attained notable attention in the scientific community (Hayta et al., 2014; Guo et al., 2017; Peterson et al., 2017; Abdelhalim et al., 2017).

Plukenetia volubilis L., "Sacha Inchi", (Euphorbiaceae family) is an oleaginous, climbing and native plant grows in the Andean region of South America. The seeds of this plant are valued for their high oil and protein content. Sacha Inchi seeds and oil have been consumed in Peru as part of the Inca diet for 3000 years (Antunez, 1981; Mcbride, 1993; Guillespie, 2007; Dosterts et al., 2009). The Sacha Inchi oil is predominantly polyunsaturated fatty acids with an unusual high content of α-linolenic acid (ω3) (40-48%), a lesser extent saturated fatty acids and very low levels of trans-fatty acids (Sathe et al., 2012). Since consumption of omega-3 fatty acids, whether from fish oil, flax or supplements, can protect against cardiovascular disease, the consumption of Sacha Inchi oil, as a novel vegetable source of omega-3 fatty acids, may become an important alternative for the prevention or protection against cardiovascular disease (Maurer et al., 2012). Studies performed for evaluating the toxicological potential these oils showed LD_{50} values of 111.65 mg/kg in mice (Cordova et al., 2006). On the other hands, Sacha Inchi powder is a vegetable protein concentrate derived from the seeds of this plant showing promissory beneficial effects in humans. However, no studies have been conducted for describing the potential toxicological effects of the Sacha Inchi powder obtained from *Plukenetia volubilis* L. plant growing in Peru. Meanwhile, toxicological studies are very important to evaluate the safety of food products for future human use. Thus, at present work the toxicological potential of the Sacha Inchi powder through of the conduction of oral acute and repetitive studies in rodents, as well as its possible genotoxic effects, measured using micronucleus assay, was evaluated.

MATERIAL AND METHODS

Chemicals

Carboxymethyl cellulose, May-Grunwald, giemsa, hematoxylin, eosin, acacia gum and cyclophosphamide were purchased from Sigma (St. Louis, M.O., USA). The glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, urea, alkaline phosphatase and creatinine kits were supplied by "C. J. Finlay" Laboratories, Havana, Cuba.

Test material

The seeds used to obtain the seed cake came from *Plukenetia volubilis* L. plant that has been domesticated in the San Martin Region, Peru. A voucher was deposited at the Herbarium of San Marcos University in Lima, Peru (No. 157-USM-2016).

For the studies, Sacha Inchi powder was provided by Amazon Health Products, Lima, Peru (sample lot SIHAGL15247). For preparing the product, the oil is removed from the seed under good manufacturing procedures using a cold press machine in a standard process, known as cold pressing. Then, the crude oil was clarified and press-filtered, the resulting defatted cake was then heat-treated, milled, cooled and packaged. Sacha Inchi powder obtained was a single ingredient product without solvents, chemicals or additives and it contains about 60% protein, 20% carbohydrates and 12% fat. These values are within the specifications indicated in the "Peruvian Technical Norm NTP 151.407.2015. Sacha Inchi and derivate. Processed protein flours. Requirements" (listed in Table 1). Protein content was measured according to methodology ISO 5983:2002 and ash content was measured according to AOAC 942.05.2012.

Animals

Mice were obtained from the "Centro para la Producción de Animales de Laboratorio" (CENPALAB, Havana, Cuba) and were acclimatized

to environmental conditions for one week before tests.

Table 1. Product specifications of Sacha Inchi powder.

Parameter	Specification
Protein	≥ 55%
Fat	≤ 13%
Ash	≤ 5.8%
Moisture	≤ 6%
Smell	Characteristic, no rancid odors
Taste	Characteristic, no rancidity
Color	Characteristic (beige to light brown)
Appearance	Homogenous powder

Peruvian Technical Standard. NTP 151.407.2015: Sacha inchi and derivate. Processed protein flours. Requirements.

Sprague Dawley rats (150-200 g) and OF1 mice (20-25 g) of both sexes were used in an acute toxicity assay. Male and female rats weighting 150 to 200 g were used in a repetitive dose toxicity assay (90 days). Animals were housed in plastic and stainless-steel grid-floored cages, which were kept at 23 ± 2°C, relative humidity 40-60%, 12 h light/dark cycles with food and water *ad libitum*. The mice and rats employed were randomly distributed for tests. A minimum number of animals were used to obtain reliable results. In the acute toxicity studies, 3 animals/sex/doses were used, and for the repetitive dose assay 12 animals/sex/groups were randomly assigned to four groups (control, low, medium and high dose group).

All studies were in compliance with Good Laboratory Practice (GLP) standards. The experiments were conducted in accordance with the ethical guidelines for investigations with laboratory animals and were approved by the Ethical Committee for Animal Experimentation of the Center of Marine Bioproducts (0011-2015), Havana, Cuba.

Acute oral toxicity study in rats and mice

Mice were fasted for 24 h prior to the commencement of this test. Six animals (mice or rats); three males and three females were used and each animal were given a single oral dose of 2000 mg/kg of the Sacha Inchi powder. Animals were observed strictly and individually for first 30 min after dosing and periodically during first 24 h (with special attention during first 4 h) and daily thereafter for 14 days. Mice were observed for altered autonomic effects (lacrimation, salivation, piloerection), central nervous system effect (tremors, convulsion, drowsiness) skin (fur), body weight, food consumption, water consumption and mortality. Body weight data were recorded at the beginning and at the end of the study. The incidence of gross pathological changes was also observed during the necropsy performed at day 14 in each study. The study was conducted according to the ATC method described in the OECD Protocol 423 (OECD, 2000). The product was suspended in carboxymethyl cellulose at 0.5% (CMC) and administered at 10 mL/kg to mice and 5 mL/kg to rats.

Repeated dose 90 days oral toxicity study in rats

The test material was administered by single oral intubations during 90 consecutive days. Four experimental groups (12 animals/sex/groups) were included: three groups were treated with Sacha Inchi powder doses of 50, 250 y 500 mg/kg/day and the control group received equivalent volumes of CMC (0.5%). During the administration period, animal general appearance was observed every day and body weight was measured weekly and at necropsy. This experiment was carried out according to OECD (1998).

At the end of the study, animals were fasted for 16 h and the necropsy day they were anesthetized under ether atmosphere. Blood samples were collected from the abdominal aorta and around 1 mL of blood was treated with EDTA for determining hemoglobin concentration (Hb) and the total and differential percentage of total leucocytes (lymphocytes, monocytes, eosinophils). Analyses were performed by using a Hematology analyzer F-850 (Sysmex Co., LTD, Roche, Germany). The differential white blood count was measured by using the Brecher method and the May-Grunwald-Giemsa staining method. Serum from blood samples were collected and the following parameters: glucose, total cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, urea and creatinine were measured by 7150 automatic analyzer (Hitachi Co., Ltd., Roche, Germany).

During necropsy, the abdominal, thoracic and cranial cavities of each animal were examined while the liver, spleen, heart, kidneys and thymus were weighed (Sartorius Universal Scale, Goettingen, Germany). Tissue samples were taken from all animals in the study. Samples came from the above-mentioned organs and from lymph nodes, pituitary gland, thyroid with parathyroid, larynx/trachea, bronchi, esophagus, stomach, small and large intestines, pancreas, penis, urinary bladder, vagina, skeletal muscle, skin, eyes, cerebrum, cerebellum and lungs. Samples were preserved in 10% buffered formaldehyde, embedded in paraffin, sectioned with a rotary microtome (Leitz microtome, Wetzlar, Germany), stained with hematoxylin and eosin and examined by light microscopy. An Olympus BH2 microscope (Olympus Optical Co., Ltd. Tokyo, Japan) was used for these observations.

Micronucleus test in mice

Animals received a 2000 mg/kg single oral dose of Sacha Inchi powder, which is well tolerate under our experimental conditions and it is considered limit for this kind of studies (OECD, 1997). A control group was included, which received only CMC and it was used as negative control. Another group of animals, used as positive control group, received 100 mg/kg of cyclophosphamide (CP) by a single intraperitoneal injection, and provided information about the validity of the experimental design. Each group included 6 animals. Bone marrow from the femora was down 48 h after administration for Sacha Inchi powder treated, vehicle or single injection for CP (positive control).

Both femora bones were removed from sacrificed animals and bones were freed from muscle by gentle traction. The distal epiphyseal portion was torn off together with the rest of the tibia and the surrounding muscle. The proximal end of the femur was carefully shortened until a small opening to the marrow canal became visible. Then, 2 mL of serum were introduced into the bone canal and the femur was submerged in a centrifuge tube filled with fetal calf serum. The marrow was aspirated and flushed for several times. Cells were centrifuged at 1000 rev/min for 5 min. Two drops of cell suspension were placed onto clean, dry slides and smeared, fixed in methanol and stained with giemsa 5% (v/v) for 12 min. The presence of micronuclei and the percentage of cells containing micronucleus were determined in a sample of 2000 polychromatic erythrocytes (PCE). Normochromatic erythrocytes (NCE) were also scored in 200 erythrocytes samples to determine the PCE/NCE ratio (Krishna and Hayashi, 2000).

Statistical analysis

Means and standard deviations of body weight, food consumption, hematology, blood chemistry, relative organ weights and micronucleus data were determined. The statistical analysis was performed by one-way ANOVA using the GraphPad Prism 5 statistical software package. Homoscedasticity was assessed by the Bartlett test for homogeneity of variances. In the absence of variance homogeneity, the Kruskal–Wallis test was used. Data was expressed as mean ± SD and the statistical significance p level used was 0.05. Histopathology analysis was done by Fisher test.

RESULTS

Oral acute studies in rats and mice

All animals survived the 14-day observation period after oral administration of 2000 mg/kg of Sacha Inchi powder and their body weight gain increased like controls ($p>0.05$). During the first three hours after administration, only piloerection and grooming were observed and the following 14 days there were no toxicity signals. In addition, there were no findings of gross pathology alterations at necropsy and no significant changes were observed in any organ. Any significant changes on body weight gain were observed neither rats nor mice (data not shown). Samples taken from selected organs were macroscopic observed and no findings were found, therefore, samples were not taken for histopathological studies according with 423 guidelines (Class toxicity test), (OECD, 2000).

Repeated dose 90 days oral toxicity study in rats

No animals died during the 90 days oral toxicity study of Sacha Inchi powder in Sprague Dawley rats. Body weight gain and food consumption were

unaffected after Sacha Inchi powder exposure during the experiment. No significant differences were observed in the hematological and clinical chemistry parameters in female and male rats (Tables 2 and 3). These parameters were within normal range according to physiological background data and the historical values reported for these animals (Aleman et al., 1998).

Gross necropsy findings did not reveal changes in any organs and tissues examined. The results of the weight of organs percentage are shown in Table 4. There were no statistically significant differences between the control group and the treated groups in organ weight. Recorded values were also within the normal physiological range described for this species. A search for histopathological changes was also analyzed in the organs and tissues of controls and animals treated with the different dose levels of the product. Histological evaluation did not show any alterations in the animals, only it was observed a chronic larynx in three animals: one female from the control group, one female from the 500 mg/kg and in one male treated with doses of 250 mg/kg. Furthermore, another male was observed with perivasculitis pulmonary at doses of 500 mg/kg. Findings observed were very few and in our consideration, are not related with the administration of Sacha Inchi powder (Table 5).

Micronucleus assay in mice

The results of micronucleus assay showed no differences ($p>0.05$) between the mean of the group treated and the negative control compared with the percent of polychromatic erythrocytes for both sex after exposure of Sacha Inchi powder. There were no significant differences in micronucleus frequency between treated and control groups. As it was expected, the administration of CP significantly increased the ratios of (PCE/NCE) and PCE in both male and female animals indicated the validity of the species selected and the study design to detect *in vivo* cytotoxic and clastogenic effects (Table 6).

Table 2. Selected hematological parameter following 90 days of exposure to Sacha Inchi powder in Sprague Dawley rats.

Parameter	Doses (mg/kg/day)			
	0	50	250	500
Male				
Hemoglobin (g/L)	202 ± 18.0	196 ± 7.0	199 ± 11.0	201 ± 14.0
Leukocytes (cell/mm³ x 10³)	3.5 ± 0.5	3.1 ± 0.5	4.4 ± 0.4	4.2 ± 0.7
Neutrophils (cell/mm³ x 10³)	13.0 ± 2.7	12.9 ± 2.0	13.2 ± 1.5	12.5 ± 2.0
Lymphocytes (cell/mm³ x 10³)	85.0 ± 2.6	86.0 ± 2.0	85.0 ± 1.6	87.0 ± 2.1
Eosinophils (cell/mm³ x 10³)	0.4 ± 0.2	0	0	0.2 ± 0.1
Monocytes (cell/mm³ x 10³)	1.6 ± 0.4	2.2 ± 0.8	1.2 ± 0.4	1.2 ± 0.5
Female				
Hemoglobin (g/L)	190 ± 5.0	188 ± 6.0	191 ± 3.0	188 ± 4.0
Leukocytes (cell/mm³ x 10³)	2.8 ± 0.3	2.4 ± 0.5	3.8 ± 0.8	4.0 ± 0.8
Neutrophils (cell/mm³ x 10³)	13.7 ± 1.5	12.8 ± 1.2	14.0 ± 1.4	12.0 ± 2.0
Lymphocytes (cell/mm³ x 10³)	84.8 ± 1.4	85.1 ± 0.9	84.1 ± 1.8	86.8 ± 1.8
Eosinophils (cell/mm³ x 10³)	0.2 ± 0.1	0	0.6 ± 0.3	0.6 ± 0.3
Monocytes (cell/mm³ x 10³)	1.3 ± 0.4	1.2 ± 0.7	1.3 ± 0.6	0.6 ± 0.5

Data are presented as mean ± SD. Each group included 12 animals. No significant differences were observed among groups, Dunnett´s Test, $p>0.05$.

Table 3. Biochemical parameters following 90 days of exposure to Sacha Inchi powder in Sprague Dawley rats.

Parameter	Doses (mg/kg/day)			
	0	50	250	500
Male				
Glucose (mmol/L)	2.5 ± 0.3	2.7 ± 0.6	3.1 ± 0.2	3.0 ± 0.5
Cholesterol (mmol/L)	0.7 ± 0.09	0.6 ± 0.07	0.6 ± 0.1	0.6 ± 0.1
ASAT (U/L)	95.7 ± 6.9	105.2 ± 11.7	110.2 ± 8.8	110.9 ± 7.0
ALAT (U/L)	20.4 ± 0.6	26.7 ± 2.4	24.4 ± 3.1	22.9 ± 2.9
Alkaline phosphatase (U/L)	29.1 ± 2.3	33.1 ± 2.3	32.8 ± 2.6	28.9 ± 2.7
Creatinine (µmol/L)	46.4 ± 2.1	42.1± 2.2	45.9 ± 1.7	40.8 ± 2.8
Urea (mmol/L)	8.0 ± 0.4	8.9 ± 0.6	8.20 ± 0.7	8.90 ± 0.6
Female				
Glucose (mmol/L)	4.6 ± 0.5	4.9 ± 0.9	4.50 ± 1.0	4.5 ± 0.9
Cholesterol (mmol/L)	1.1 ± 0.09	1.2 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
ASAT (U/L)	91.4 ± 7.3	98.6 ± 6.9	84.7 ± 7.7	86.2 ± 12.0
ALAT (U/L)	19.7 ± 3.4	19.0 ± 2.6	12.6 ± 1.4	17.4 ± 3.0
Alkaline phosphatase (U/L)	36.0 ± 3.2	28.5 ± 19.9	33.3 ± 4.1	25.1 ± 19.2
Creatinine(µmol/L)	39.3 ± 3.2	41.5 ± 1.4	41.6 ± 2.7	42.4 ± 1.9
Urea (mmol/L)	8.8 ± 0.5	8.8 ± 0.8	9.5 ± 0.6	10.7 ± 0.8

Data are presented as mean ± SD. Each group included 12 animals. No significant differences were observed among groups, Dunnett´s Test, p>0.05.

Table 4. Relative organ weights of Sprague Dawley rats treated during 90 days with Sacha Inchi powder.

Organ	Doses (mg/kg/day)			
	0	50	250	500
Male				
Liver	2.38 ± 0.08	2.30 ± 0.06	2.29 ± 0.05	2.34 ± 0.05
Kidneys	0.66 ± 0.02	0.63 ± 0.02	0.67 ± 0.02	0.69 ± 0.01
Thymus	0.11 ± 0.01	0.12 ± 0.09	0.09 ± 0.01	0.10 ± 0.02
Spleen	0.17 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.16 ± 0.01
Heart	0.29 ± 0.01	0.31 ± 0.02	0.31 ± 0.01	0.29 ± 0.01
Female				
Liver	2.39 ± 0.07	2.33 ± 0.07	2.43 ± 0.09	2.35 ± 0.04
Kidney	0.61 ± 0.02	0.58 ± 0.02	0.62 ± 0.02	0.64 ± 0.01
Thymus	0.17 ± 0.02	0.13 ± 0.01	0.16 ± 0.02	0.14 ± 0.01
Spleen	0.22 ± 0.02	0.19 ± 0.01	0.20 ± 0.01	0.17 ± 0.02
Heart	0.31 ± 0.01	0.30 ± 0.01	0.31 ± 0.01	0.31 ± 0.01

Data are presented as mean ± SD. Each group included 12 animals. No significant differences were observed among groups, Dunnett´s Test, p>0.05.

Table 5. Microscopic findings observed in Sprague Dawley rats after 90 days treated with Sacha Inchi powder.

Lesions	Doses (mg/kg/day)			
	0	50	250	500
Male				
Chronic larynx	0/10	0/10	1/10	0/10
Perivasculitis pulmonary	0/10	0/10	0/10	1/10
Female				
Chronic larynx	1/10	0/10	0/10	1/10

Data are presented as mean ± SD. Each group included 12 animals. No significant differences were observed among groups, Dunnett´s Test, p>0.05.

Table 6. Frequency of micronucleus on bone marrow in OF1 mice after Sacha Inchi powder treatment.

Treatment	Sex	EPCMN/1000 EPC	EPC/EPC+ENC
Negative control	Male	0.17 ± 0.04	0.90 ± 0.10
	Female	0.19 ± 0.10	1.14 ± 0.10
Sacha inchi powder	Male	0.15 ± 0.04	0.73 ± 0.26
	Female	0.18 ± 0.10	1.09 ± 0.16
Cyclophosphamide	Male	4.67 ± 0.87*	3.60 ± 2.29*
	Female	6.48 ± 2.92*	4.17 ± 1.51*

Data are presented as mean ± SD; EPC: polychromatic erythrocytes. EPCMN: Micronucleus polychromatic erythrocytes, ENC: normochromatic erythrocytes. *significant different compared with the negative control (p<0.05).

DISCUSSION

Acute toxicity studies are the first battery of tests conducted in a preclinical toxicological evaluation of a product for human use. The present study evaluated the oral acute toxicity in rodents of the product Sacha Inchi powder obtained from *Plukenetia volubilis* L. plant. The results showed this product did cause any mortality, toxic sign symptoms neither rats nor mice. All the animals treated with Sacha Inchi powder survived beyond 14 days observation period. Cordova et al. (2006) reported that oral acute doses of Sacha Inchi oil was innocuous to mice, but in that study dose-dependent mortality was observed, with values of LD_{50} in the order of 111.65 mg/kg. These findings could seem contradictory to present results, nevertheless, the mortality observed was associated to the presence of several diarrheas in the treated animals and the decrease of body weight, as consequence of the oil formulation tested in this study.

The repeated-dose toxicity test conducted on rats for 90 days using the Sacha Inchi powder provided data regarding the cumulative toxic effects on target organs. In general, an increase or decrease in the body weight of an animal has been used as an indicator of an adverse effect of drugs and chemicals (Teo et al., 2002). Moreover, the relative organ weight indicates whether the organ has been exposed to injury or otherwise. Impaired organs often have abnormal atrophy (Wang et al., 2007). In the present study, the relative organ weights of all treated male and female rats did not differ significantly (p>0.05) from those of the control groups. The analysis of blood parameters is relevant to risk evaluation as changes in the hematological system have a higher predictive value for human toxicity when the data are translated from animal studies (Olson et al., 2000). In terms of hematological parameters, neither those of male nor female treated rats appeared to be significant effected compared to controls. These results indicated that Sacha Inchi powder does not interfere in the formation of erythrocytes and leukocytes nor does it cause microcytosis or macrocytosis. Hence, there were no significant alterations in the hematological parame-

ters. Furthermore, no related histopathological changes were observed.

ALT and AST are important serum enzymes in the human liver and monitoring their concentrations usually help to detect chronic liver diseases (Burger et al., 2005). ALT is a cytoplasmic enzyme that is found at a very high concentration in the liver and an increase in the level of this specific enzyme suggests hepatocellular damage (Tennekoon et al., 1991). AST is an enzyme that is present in high quantity in the cytoplasm and mitochondria in different tissues, including the liver, heart, skeletal muscle, kidney and brain (Evan, 2009). In the present study, there were no statistically significant differences in AST and ALT levels between control and treated animals at any dose suggesting Sacha Inchi powder does not cause hepatic toxicity. These findings were further confirmed by histopathological examination of the liver of treated and control rats, showing normal lesions (Table 5).

Kidney function was evaluated by means of serum urea and creatinine levels. Serum creatinine, which results from the catabolism of creatine phosphate in skeletal muscle, increases when renal function is poor and decreases with the loss of skeletal muscle (Tortora and Derrickson, 2009). Hence, elevated blood creatinine is a reliable indicator of a negative impact on kidney function or impaired glomerular, these parameters were not changed during the study (Table 3).

Sacha Inchi oil is rich in fatty acids omega 3 (about 48%) and omega 6 (36%), and it has shown to reduce cholesterol levels in rats and human subjects. Gorriti et al. (2010) studied oral toxicity of Sacha Inchi oil in rats and showing that there was no toxicity at 60 days of exposure and that the administration of Sacha Inchi oil lowered the levels of cholesterol, triglycerides and increased the HDL levels in comparison with the control group. Furthermore, Gonzales et al. (2014) showed that Sacha Inchi oil lowers cholesterol levels after single oral doses of Sacha Inchi and sunflower oil in humans. Sacha Inchi powder contains about 13% of Sacha Inchi oil, since this is lower quantity it could be the reason why the levels of cholesterol in the present study were no modified (Table 3).

At last, Ruiz et al. (2013) determined the composition, amino acid profile, fatty acid profile and antinutrients contents of Sacha Inchi seed and cakes (cake is a sub-product in the extractions processes of the oil), and showing the presence of low levels of saponins and tannins in this product, which could explain at least in part the absence of toxicity.

The micronucleus is a simple cytogenetic assay based on scoring micronuclei in actively dividing cell populations. It is also a quantitative measure of chromosome damage and acts like an index of genomic damage (Hayashi et al., 1994). In this study, the exposition with the product to mice during 48 hours by oral route did not significantly increase the frequency of PCE (data were presented as percent of micronucleus in PCE) compared to control groups. This result reflects that oral administration of up to 2000 mg/kg of Sacha Inchi powder did not induce genotoxic effects in mice. Furthermore, no clinical signs of toxicity were observed during treatment or in the macroscopic analysis after animal sacrifice. Therefore, the product did not exhibit cytotoxic activity in this assay, determined as PCE/NCE ratio. As it was expected, the administration of CP (positive control) significantly increased PCE/NCE ratio and PCE in animals, thus indicating the validity of the experimental design and the species selection for detecting *in vivo* cytotoxic and clastogenic effects (Table 6). Finally, the results of this test indicated that Sacha Inchi powder had no clastogenic potential over dose evaluated.

CONCLUSIONS

From this study, no acute oral toxicity effects after 2000 mg/kg of Sacha Inchi powder administered to rodents was observed. Neither toxicity attributable to oral doses of the product up to 500 mg/kg during 90 days to rats were found. In addition, the micronucleus assay revealed that product showed no clastogenic in mice. Accordingly, the results suggest that Sacha Inchi powder is a safe and non-toxic under experimental conditions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Author contribution:

Contribution	Rodeiro I	Remirez D	Flores D
Concepts or ideas	X		X
Design	X		
Definition of intellectual content	X		
Literature search	X	X	X
Experimental studies	X	X	
Data acquisition	X	X	
Data analysis	X	X	
Statistical analysis	X	X	
Manuscript preparation	X	X	X
Manuscript editing	X		
Manuscript review	X	X	X

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