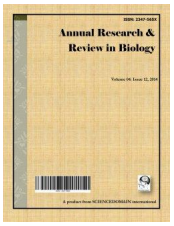

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Antimutagenic Effect of *Plukenetia volubilis* (Sacha inchi) Oil in BALB/c Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JLAA, RCA and AAG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OHC and EER managed the analyses of the study. Authors MCF and BPO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Oils with high levels of omega-3 are being commercialized as the natural supplement to avoid serious consequences related to metabolic syndrome. Sacha inchi (*Plukenetia volubilis*) oil is a natural product used as a nutraceutical in Peru. Otherwise, genotoxicity is the main test for assessing the toxicity of drugs, food and other substances. Sacha inchi is known as an oil with high content of omega-3 and others polyunsaturated fatty acids. The objective of this study was to determine the antimutagenic effect of *Plukenetia volubilis* (Sacha inchi) oil in BALB/c mice.
Study Design: Sacha inchi oil was obtained using standardized methods in order to determine its antimutagenic effect in BALB/c mice by using micronucleus test, according to the Organization of Economic Co-operation and Development (OECD) guidelines.
Place and Duration of Study: Laboratory of Experimental Pharmacology, Faculty of Medicine, Universidad Nacional Mayor de San Marcos, Lima, Peru, from January to February 2017.
Methodology: A total of 100 Balb/C albino mice (20 - 30 g) of male sex were randomly divided into five groups (n = 20). The groups were normal saline group (NS), cyclophosphamide group (CP; 40 mg/kg i.p.) and the three other groups received cyclophosphamide and Sacha inchi oil of concentrations 10, 100 and 1000 mg/kg respectively. The substances were administered three times during 24 hours. The genotoxicity in mice was evaluated determining micronucleus levels in blood and bone marrow.
Results: CP group showed higher micronucleus levels in blood and bone marrow compared with Sacha inchi oil 10, 100 and 1000 mg/kg groups (ANOVA Test $P < 0.001$ Scheffe's Post Hoc $P < 0.001$).
Conclusion: In our findings, Sacha inchi oil was not mutagenic under experimental conditions.

Keywords: Sacha inchi; toxicity; genotoxic; crude oil; cyclophosphamide; mutagenicity; nutraceutical.

ABBREVIATIONS

P. volubilis : *Plukenetia volubilis*
CP : *Cyclophosphamide*
NS : *Normal Saline*

1. INTRODUCTION

Plants have important effects due to the presence of secondary metabolites of the different chemical composition such as terpenes, steroids, flavonoids, alkaloids, anthocyanins, saponins, phenolic compounds, tannins, and quinones. Their medicinal value is based on their pharmacological action on the human and animal organism. However, these substances can also have toxic effects when they are consumed in a large or short period of time for any pathological condition [1,2].

It is known that 80% of the world population relies on using plants to alleviate the pain of some illnesses. Therefore, the WHO (World Health Organization) insists that the use of medicinal plants can be of great use to primary health care systems, but based on scientific evidence that shows the safety, effectiveness and quality required for human consumption [3,4].

Sacha inchi (*Plukenetia volubilis* L), which belongs to the family Euphorbiaceae has been historically used in the Peruvian Amazon, is known as Sacha inchi, inca peanut, mountain peanut, sacha peanut, sachainche, Fragariopsis, Supua (Bolivia), Amauebe, Amui-or yachisacha, sacha Yuchi, sachayuchiqui, sampannankii, suwaa, etc. It was used as food source and cosmetics by the Amazonian ethnic groups and later, it was used by rural and non-rural populations [5,6].

Sacha inchi oil derives from the cold pressing of its seeds. This oil has triglycerides, polyphenols and tocopherols (mainly γ-tocopherol). It is one of the best plant sources of omega-3 fatty acids (45-55%) and the richest in unsaturated fatty acids (35 -60%) [7]. It has beneficial effects on the lipid profile in patients with dyslipidemia [8,9], but the evaluation of its efficacy and safety in randomized clinical trials is required [10].

There is little research about the toxicity of Sacha inchi. One study showed that oral administration for 60 days in mice, with LD50 above 37 g / kg body weight in mice is innocuous [11]. This study aims to evaluate the antimutagenic effect of the oil of *Plukenetia volubilis* L. (Sacha inchi) in Balb/C mice.

2. MATERIALS AND METHODS

2.1 Chemicals

Chemicals were of analytical grade and purchased from Merck S.A and Medifarma S.A. (Lima, Peru).

2.2 Animals

Balb/C male albino mice with the weight of 25–30 g of 8 weeks of age were used in the mutagenicity test. Animals were purchased from the National Institute of Health (NIH-Peru), Lima, Peru. All animals were housed under standard laboratory conditions [(22± 2)ºC] with access to balanced food and water *ad libitum*. Experimental protocols were carried out and followed the Guide for the Care and Use of Laboratory Animals of the National Institute of Health (NHI-Peru) and the National University of San Marcos (Faculty of medicine: Protocol Nº 0156-2014.

2.3 Oil Collection Source

Extra virgin Sacha inchi oil with the brand Ecoline ™ (Organic Sacha inchi oil) was brought in a pharmacy in the city of Lima, Peru.

2.4 Mutagenicity Assay

Oral mutagenicity of Sacha inchi (*Plukenetia volubilis* L.) oil was assessed in BALB/c mice. According to OECD 474 [12] and Schmid [13, 14], A total of 100 Balb/C albino mice (25 ± 5 grams) of male sex were randomly divided to five groups (n = 20), they were kept in the vivarium of the Faculty of Medicine of the Universidad Nacional Mayor de San Marcos (Lima, Peru). The experiment began with a 48-hour pre conditioning period. Mice were given *ad libitum* access to food and water, 12 hours' light / dark cycle, temperatures between 22-26 ° C and 60% ± 10% of relative humidity.

During the entire experimental process, international ethical principles for research using laboratory animals were respected [15]. The animals were divided into five groups of twenty mice each: the control group received 10 mL/kg of normal saline (NS), cyclophosphamide group (CP) received cyclophosphamide 40 mg / kg i.p. and the three other groups (SI10, SI100, SI1000 by oral administration) received cyclophosphamide and Sacha inchi oil of concentrations 10, 100 and 1000 mg/kg respectively. The animals were orally exposed to the substances, three times during 24 hours with intervals of 8 hours. Immediately, mice were placed into a glass chamber where they became anesthetized with ether. Peripheral blood was obtained by cardiac puncture to prepare blood film which allows evaluating the micronucleated polychromatic erythrocyte (MNPCE). Then, mice were euthanized by intraperitoneal injection of sodium pentobarbital 100 mg/kg.

Gently flushing the marrow cavity with 3 mL of fetal bovine serum (FBS), the femoral bone marrow was extracted. It was centrifuged at 1000 rpm for 10 minutes. Once the supernatant was removed, the cell button was dispersed over slides. The slides (minimum 2 per animal) were left to dry at room temperature for 24 hours. They were fixed with absolute methanol for 5 minutes and stained with 5% Giemsa stain for 12-15 minutes. The slides were coded and examined by two independent observers (blind) under Olympus BH-2 microscope (100x oil immersion lens). The frequency of micronucleated polychromatic erythrocyte (MNPCE), based on the observation of 1000 polychromatic erythrocytes (PCE) per animal, was recorded [16].

2.5 Statistical Analysis

Numerical variables were described with measures of central tendency and dispersion, mean and standard deviation, respectively. The normality and homogeneity of variance were evaluated using the Shapiro-Wilkt´s test. Subsequently, an analysis of variance (ANOVA) with Scheffe´s Post Hoc was used with variables that showed significant difference ($p < 0.05$) between the groups. All statistical analysis was performed using SPSS V 24.0 software.

3. RESULTS AND DISCUSSION

3.1 Determination of Genotoxicity

The genotoxicity in mice was evaluated by determining micronucleus levels in the blood. The CP group showed 13.20 ± 0.22 micronuclei. The level of micronucleus was higher in CP group compared with the groups that received 10, 100 and 1000 mg / kg of Sacha inchi oil (ANOVA Test $p < 0.001$ Scheffe´s Post Hoc $p < 0.001$). The inhibition of micronuclei was higher than 50% for both the group of 100 and 1000 mg / kg of Sacha inchi oil (Table 1 and Fig. 1).

The evaluation of genotoxicity in mice by determining micronucleus levels in bone marrow showed 1.80 ± 0.14 in the CP group. The level of micronucleus was higher in CP group compared with the groups that received 10, 100 and 1000 mg / kg of Sacha inchi oil (ANOVA Test p <0.001 Scheffe´s Post Hoc p <0.001). The inhibition of micronuclei was higher than 60% for both the 10 and 100 mg / kg of Sacha inchi oil (Table 2 and Fig. 2).

3.2 Discussion

Genetic toxicity or mutagenicity is the process made by an agent that induces damage to DNA and other cellular targets that control the integrity of genetic material [17]. The assessment of mutagenicity using micronucleus in blood and bone marrow of mammals is used to detect lesions in chromosomes or mitotic apparatus of erythroblasts. Erythrocytes took from the bone marrow and/or peripheral blood of animals (usual rodents) are analyzed to determine chromosomal lesions in a living system. Genotoxic substances cause cytogenetic damage leading to the formation of micronuclei containing *chromosomal fragments* and whole *lagging chromosomes* [18]. When the erythroblasts of the bone marrow became polychromatic erythrocyte, the main nucleus is expelled and the micronucleus remains in the cytoplasm, otherwise the cell would be anucleated. Because of the lack of the main nucleus, is easier to visualize the micronuclei in these cells. An increased micronuclei frequency in polychromatic erythrocytes is indicative of the existence of induced chromosome damage [12,16].

Studies with cyclophosphamide demonstrate an intimate relationship between chromosomal damage and teratogenic effects caused by this substance in fetal liver [19,20], during pregnancy in mice [21,22]. The efficient use of cyclophosphamide has been demonstrated as a positive control in the assays of teratogenicity in rats and mice for their clastogenic and aneugenic effects [23,24]. Figs. 1 and 2 shows the toxicity of cyclophosphamide to evaluate the antimutagenicity of Sacha inchi oil in erythrocytes of blood and bone marrow. The CP values were within 13.20 and 1.80 micronuclei per 1000 polychromatic erythrocytes (PCE) respectively, these results are seemed according to Arroyo et al. [20], previous studies related to genotoxicity of *Chuquiraga spinosa.* Cyclophosphamide is an alkylating agent which reparative mechanisms have ruptures effects formatting monoadducts and cross-links between chains. This delays the anaphasic onset in somatic cells [25,26].

Table 1. Number of micronuclei obtained to evaluate the genotoxicity of Sacha inchi oil in erythrocytes of the blood of *Mus musculus* (OECD 474)

Treatment	Average	Standard error	95% confidence intervals		%IM
			Lower limit	Upper limit	
Normal saline	0.20	0.09	0.01	0.39	----
Cyclophosphamide (CP) 40 mg/Kg.	13.20 [a, b ,c]	0.22	12.73	13.67	0.00
CP + Sacha inchi 10 mg/Kg.	11.95[a]	0.33	11.26	12.64	9.47
CP + Sacha inchi 100 mg/Kg.	6.30[b]	0.16	5.96	6.64	52.27
CP + Sacha inchi 1000 mg/Kg.	4.80[c]	0.42	3.92	5.68	63.64

*% IM = Inhibition of Micronuclei = [((CP-NS)/CP)*100], CF: Cyclophosphamide, SI: Sacha inchi oil, ANOVA Test p <0.001 Scheffe´s Post Hoc a= p < 0, 001, b= p < 0, 001 y c =p < 0,01*

Table 2. Number of micronuclei obtained to evaluate the genotoxicity of Sacha inchi oil erythrocytes of the *Mus musculus* (OECD 474)

Treatment	Average	Standard error	95% confidence intervals		%IM
			Lower limit	Upper limit	
Normal saline	0.10	0.10	-0.11	0.31	----
Cyclophosphamide (CP) 40 mg/Kg.	1.80 [a, b ,c]	0.14	1.51	2.09	0.00
CP + Sacha inchi 10 mg/Kg.	0.65 [a]	0.11	0.42	0.88	63.89
CP + Sacha inchi 100 mg/Kg.	0.60 [b]	0.11	0.36	0.84	66.67
CP + Sacha inchi 1000 mg/Kg.	1.00 [c]	0.00	1.00	1.00	44.44

*% IM = Inhibition of Micronuclei = [((CP-NS)/CP)*100], CF: Cyclophosphamide, SI: Sacha inchi oil, ANOVA Test p <0.01 Scheffe´s Post Hoc a= p < 0, 001, b= p < 0, 001 y c =p < 0,001*

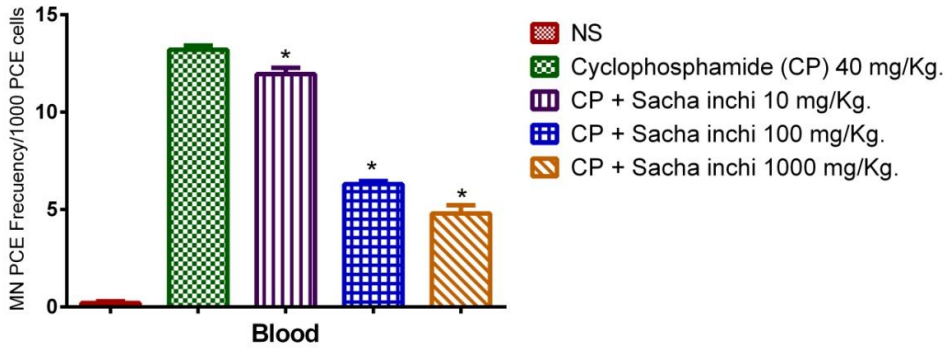

Fig. 1. Effect of Sacha inchi (*Plukenetia volubilis*) oil on the frequency of micronucleated polychromatic erythrocytes (MNCPE) in blood

*Each value represents mean ± DS (n=20); ANOVA followed by Scheffe`s post hoc test *(p< 0.001) versus group CP*

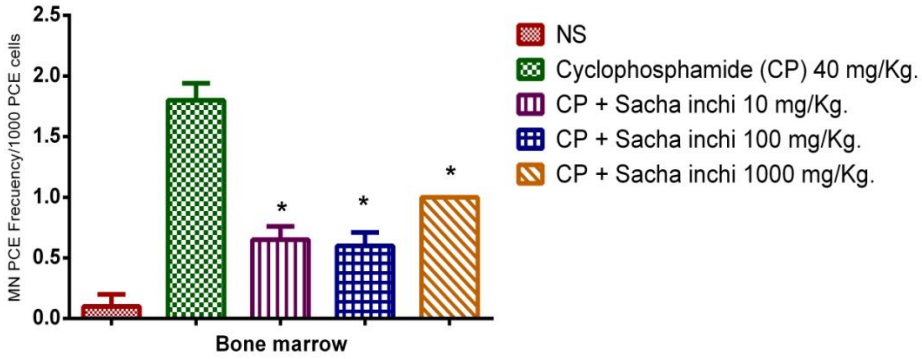

Fig. 2. Effect of Sacha inchi (*Plukenetia volubilis*) oil on the frequency of micronucleated polychromatic erythrocytes (MNCPE) in bone marrow

*Each value represents mean ± DS (n=20); ANOVA followed by Scheffe`s post hoc test *(p< 0.001) versus group CP*

Vizoso-Parra et al. [27] evaluated the genotoxicity of extracts of *Plantago lanceolata L.* and *Matricaria recutita L.* and found that their components did not have an effect on hereditary processes and the observed effects may be due to the presence of antimutagenic substances. Nigro et al. [28], evaluated the extract of *Aloysia citriodora* and concluded that it did not have cytotoxicity or genotoxicity effect due to the absence of active ingredients that cause any damage to single or double stranded DNA. The presence of polyphenols [29] and monoterpenes [30], and their antioxidant activity would stimulate the elimination of free radicals suppressing the genotoxicity of chemical agents [31,32].

The genotoxicity assessment in blood micronucleus (Fig. 1) shows a greater decrease in the average number of micronuclei in the group that received cyclophosphamide 40 mg / kg and Sacha inchi oil 1000 mg / kg. Its inhibition percentage was 63.64% of the one of the group that received only cyclophosphamide 40 mg / kg. The genotoxicity assessment of bone marrow shows greater decrease in the average number of micronuclei in the group that received cyclophosphamide and Sacha inchi oil 100 mg / kg, which showed an inhibition of 66.67% (Fig. 2). The presence of phytocompounds as monounsaturated (ω-9), polyunsatured (ω-3 and ω-6) and other fatty acids (malvalic, sterculic and abietic), terpenes, steroids, β-carotene, chlorophyll, and flavonoids [33] could explain the results which they are found in Sacha inchi seeds. Furthermore, antioxidants as polyunsatured fats [34,35,36] interrupt radical chain *oxidation by* donating *a* hydrogen *atom* (H)

to quench *lipid* peroxyl *radicals* and becoming another more stable radical. This new radical does not react with lipids but with other similar molecules; generating a non-radical product or quinones, which can be considered antioxidants, after undergoing further oxidation [37].

4. CONCLUSION

Based on the results, the present study concludes that Sacha inchi oil has antimutagenic effect at the concentrations tested. Observational studies are needed to quantify the actual consumption of Sacha inchi oil among human population prior to experimental studies The decrease in the level of micronucleus in the different experimental groups demonstrates that Sacha inchi oil is not mutagenic in BALB/c albino mice.

ETHICAL APPROVAL

As per international standard or university standard (The National University of San Marcos, Faculty of medicine: Protocol Nº 0156-2014) was written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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