Pharmacogn J. 2021; 13(5): 1287-1296
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Antioxidant Capacity of *Chuquiraga Spinosa* Less. "Huamanpinta" and Prevention of Carrageenan-Induced Inflammation in Mice

Hugo Jesús Justil-Guerrero[1,2], Jorge Luis Arroyo-Acevedo[1,2], Juan Pedro Rojas-Armas[1,2], Miriam Palomino-Pacheco[1], Magaly Villena-Tejada[3,*], Wilmer Atilio Segura Vílchez[4]

ABSTRACT

Objective: To evaluate the antioxidant capacity of *Chuquiraga spinosa* extracts and prevention of carrageenan-induced inflammation in mice. **Methodology:** Experimental design: plant species, erythrocytes and male BALB C53 mice, were considered as biological material. Antioxidant capacity was evaluated in 50%, 70%, 96% and aqueous ethanolic extracts by 2,2-Diphenyl-1-Picrylhydrazyl reduction, malondialdehyde inhibition in oxidized erythrocytes with H_2O_2 and correlating with polyphenol content equivalent to gallic acid/gram dry extract. Inflammation was evaluated by inoculating carrageenan 2% in "subcutaneous air bag" of mice: 1) White, 2) carrageenan, 3) dexamethasone 2 mg/kg, 4-6) ethanolic extract 70% doses 100, 250 and 500 mg/kg respectively; determining nitric oxide, malondialdehyde, total proteins, albumin, leukocytes in exudate and histological changes. **Results:** Alkaloids, flavonoids, terpenes, phenolic compounds, tannins, carbohydrates, triterpenes, steroids and sesquiterpene lactones were identified; aqueous extract presented greater reduction of 2,2-Diphenyl-1-Picrylhydrazyl (CI50 = 58.99 μg/mL), ethanolic extract 70% presented greater inhibition of malondialdehyde in erythrocytes (CI50 = 16.44 nm/mL); It was observed that the higher the amount of polyphenols, the greater the reduction of 2,2-Diphenyl-1-Picrylhydrazyl (r=-0.909) and the greater the inhibition of malondialdehyde (r=-0.781). With 500 mg/kg of 70% ethanolic extract there was greater anti-inflammatory effect inhibiting malondialdehyde, nitric oxide, albumin, total proteins and leukocytes in 55.55%, 81.92%, 41.20%, 31.51% and 32.45% (p<0.01) respectively and less infiltration of leukocytes and lymphocytes in air sac membrane. **Conclusion**: The extracts of aerial parts of *Chuquiraga spinosa* showed antioxidant capacity correlated to polyphenol content. The 70% ethanolic extract prevented inflammation in mice in a dose-dependent manner.

Key words: Oxidative stress, Antioxidant, Ethanolic extract, Lipoperoxidation, Nitric oxide, Leukocytes.

Hugo Jesús Justil-Guerrero[1,2], Jorge Luis Arroyo-Acevedo[1,2], Juan Pedro Rojas-Armas[1,2], Miriam Palomino-Pacheco[1], Magaly Villena-Tejada[3,*], Wilmer Atilio Segura Vílchez[4]

[1]*Pharmacology Laboratory of the Faculty of Medicine of the Universidad Nacional Mayor de San Marcos. Lima, PERÚ.*
[2]*Clinical Research Institute of the Faculty of Medicine of the Universidad Nacional Mayor de San Marcos. Lima, PERÚ.*
[3]*Academic Department of Pharmacy, Faculty of Health Sciences, Universidad Nacional de San Antonio Abad del Cusco. Cusco, PERÚ.*
[4]*Management of Forensic Thanatology of the Institute of Legal Medicine and. Forensic Sciences. Public Prosecutor's Office. Lima, PERÚ.*

Correspondence

Magaly Villena-Tejada

Academic Department of Pharmacy, Faculty of Health Sciences, Universidad Nacional de San Antonio Abad del Cusco. Cusco, PERÚ.

Phone number: +51984936267

E-mail: magaly.villena@unsaac.edu.pe

History

- Submission Date: 14-06-2021;
- Review completed: 11-07-2021;
- Accepted Date: 20-07-2021.

DOI : 10.5530/pj.2021.13.163

Article Available online

http://www.phcogj.com/v13/i5

Copyright

© 2021 Phcogj.Com. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

INTRODUCTION

Oxidative stress is the imbalance between the antioxidant system and free radicals derived from oxygen and nitrogen that cause cellular aging[1]. These radicals produce lipoperoxidation and can damage tissue cells producing chronic inflammatory diseases[2]. Erythrocytes in their structure have a high content of polyunsaturated fatty acids, oxygen and ferrous ions in the ligand state that can react with free radicals[3] generating lipoperoxidation by breaking double bonds of polyunsaturated fatty acids leading to the formation of isoprostanes, malondialdehyde (MDA), 4-Hydroxynonellal[4], which can alter the physiological functions of the cell membrane such as cell deformation, fluidity, permeability and alteration of the functional integrity and structure of the cell[5]; being responsible for DNA damage by mutagenesis, being directly involved in the pathophysiology of several chronic diseases such as cancer[6], diabetes[7] and lung diseases[8]. Chronic inflammation is characterized by the migration of T lymphocytes and macrophages in the affected area inducing the production of Cyclooxygenase 2 (COX-2), which is associated with increased cell proliferation and reduced apoptosis[9].

The plant species *Chuquiraga spinosa* Less (ChS) belongs to the asteraceae family and is traditionally used by the population in the treatment of blennorrhagia, as a diuretic and to alleviate bronchial affections[10]. In its structure, glycosylated flavonoids have been identified (quercetin-3-O-glucuronide, quercetin-3-rutenoside, quercetin-3-O-glucoside, kampherol 3-O-rutenoside, kaempherol-3-O-glucuronide, kaempherol-3-O-glucuronide, isorhamnetin-3-O-glucuronide, isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucoside)[11] and phenolic acids[12]. The flavonols kaempferol, quercetin and isorhamnetin inhibit the expression of induced nitric oxide synthase (iNOS), decreasing the production of nitric oxide (NO), as well as inhibiting the activation of NF-kB[13]. The ethanolic extract of the aerial parts of ChS showed antioxidant, cytotoxic[14] and chemopreventive activity in induced prostate cancer in rats[15]. The evaluation of the methanolic extract showed antioxidant activity and anti-inflammatory effect in subplantar edema and ear inflammation in mice[12] and the chloroformic extract showed antioxidant, immunomodulatory and anti-inflammatory activity in subplantar edema in rats[16].

The carrageenan-induced air sac inflammation model on the dorsum of rodents allows the evaluation of biochemical parameters in the exudate as well as histological changes in the structure of the air sac membrane[17]. Considering that the pharmacological effects of ChS at the biochemical and histological level in models of inflammation are still unknown, this study increases the knowledge of the effect of ChS on one part of the inflammation

Cite this article: Justil-Guerrero HJ, Arroyo-Acevedo JL, Rojas-Armas JP, Palomino-Pacheco M, Villena-Tejada M, Segura-Vilchez WA. Antioxidant Capacity of *Chuquiraga Spinosa* Less. "Huamanpinta" and Prevention of Carrageenan-Induced Inflammation in Mice. Pharmacogn J. 2021;13(5): 1287-1296.

Phcogj.com

process. On the other hand, in the process of extraction of secondary metabolites, it is observed that the solvent with the highest antioxidant and lipoperoxidation inhibition capacity has not yet been standardized. Therefore, this research aims to contribute to the knowledge of the solvent with the highest antioxidant and lipoperoxidation inhibitory capacity in erythrocytes and the evaluation of the anti-inflammatory effect of the ChS extract with the highest antioxidant activity in a model of inflammation induced with carrageenan in an air bag in mice.

MATERIALS AND METHODS

The research design is experimental, it was developed following the guidelines of the "ARRIVE" guide for research with experimental animals. In the population, the plant species *Chuquiraga Spinosa* Less, the red blood cell package (PGR) was considered. The latter was obtained by cardiac puncture in 4 male Holtzman strain rats weighing 200 to 220 g. For the evaluation of the prevention of inflammation, 36 male BALB/C53 strain mice weighing 25 to 30 g were used. The units of analysis were ChS extracts at different concentrations, erythrocytes subjected to oxidative stress and mice with carrageenan-induced inflammation.

Handling of experimental animals

The rats and mice were obtained from the Instituto Nacional de Salud (INS) Lima, Peru. For the handling of the animals, the indications of the "Guide for the care and use of laboratory animals" of the National Research Council[18] were considered in accordance with the animal protection law (Law 27265). The principles considered during the experimental processes with the animals were replacement, reduction and refinement (3R) where the minimum number of rats or mice per group and standardized experimental models were considered. The personnel in charge of the care of the animals was previously trained and the infrastructure allowed the adequate cleaning of the environment where the controlled temperature was 22°C ±2°C with relative humidity between 45% and 66%. Twelve hours of light and 12 hours of darkness and noise less than 20 kHz were maintained. The animals were housed in cages that allowed them to develop 5 freedoms (thirst, hunger, fear, anxiety and distress). Feeding was balanced by providing them with rodent food acquired from the Universidad Agraria La Molina, as well as fresh water free of pathogenic microorganisms, the cage and drinking troughs were kept clean and disinfected.

Collection, taxonomic identification of the plant species

The plant species was collected in Peru, in the department of Huancavelica, Tayacaja province, Acostambo district, Parco Villanueva annex. The species was identified according to the Cronquist Classification System (1988) at the Natural History Museum of the Universidad Nacional Mayor de San Marcos.

Extraction and identification of secondary metabolites from plant species

It was carried out according to the United States Pharmacopoeia[19], washed with potable water, dried at 40°C in a temperature controlled oven, then macerated with ethanol at concentrations of 50%, 70% and 96% in a proportion of 1:10 (w/v) for 7 days and an infusion with water at 100°C, then the solvent was evaporated in a rotary evaporator; finally it was taken to desiccation in an oven at a controlled temperature of 40°C +/- 2°C, obtaining a dry extract, which was stored under refrigeration until the time of its use for the programmed assays. The identification of secondary metabolites was carried out according to Lock's method[20], using color change and precipitate assays.

Ability to reduce 2,2-Diphenyl-1-Picrylhydrazyl (DPPH)

The reduction of the DPPH radical was evaluated according to the decrease of the violet color measured with the absorbance value according to Prieto's method[21]. A 0.4 mmol solution of DPPH radical was prepared in HPLC grade methanol. The extracts were diluted in 5 concentrations in final volumes of 100 µL in a microplate to which 100 µL of the DPPH solution was added considering a group to which only DPPH radical solution was administered (control), a group only with solvent (Blank) and a group with ascorbic acid (AA), then incubated at 25°C for 30 minutes, finally proceeded to the measurement of absorbance at 517 nm using a microplate reader. To compare the reduction capacity of the extracts, the inhibitory concentration 50 (IC50) value expressed in µg/mL was used.

Total polyphenol content with Folin-Ciocalteu reagent (RFC)

It is based on the reducing power of phenols in alkaline solution (pH = 10), which are able to reduce the reagent molybdotungsten phosphate (yellow color) to molybdotungsten phosphate (blue color). The process was developed according to Quispe-Mendoza et al[22]. A calibration curve was made with gallic acid (GA) with concentrations of 1, 2, 3, 3, 4 and 5 µg/mL, then the samples of the extracts (25 mg), were diluted until obtaining concentrations of 100 µg/mL, 500 µL was taken and 250 µL of 1.2 N RFC reagent was added, shaken for 5 minutes and 1250 µL of calcium carbonate was added, after a resting time of 60 minutes, absorbance was measured with a UV-VIS spectrophotometer at 760 nm. The results were expressed in milligram equivalents of gallic acid per gram of dry extract (mg GA/g).

Determination of malondialdehyde (MDA) in erythrocytes subjected to oxidative stress

The determination of MDA in erythrocytes subjected to oxidative stress with H_2O_2 was performed according to Stock and Dormandy[23]. Blood was obtained by cardiac puncture from 4 fasted male rats using heparinized tubes. It was centrifuged at 2000 x g for 10 minutes, plasma was removed leaving PGR, which was washed 3 times with phosphate buffered saline (PBS) pH 7.4, then diluted to 20% with PBS.

From ChS96, ChS70, ChS50 and ChSAc extracts, groups of 3 samples were formed with concentrations of 100, 50, 25 and 12.5 µg/mL for each type of extract, the samples were diluted in PBS with 0.01% w/v ascorbic acid, 1 control group and 1 H_2O_2 group were considered. In 1 milliliter of each sample, H_2O_2 and control groups, 1 mL of red blood cell dilution was added, incubated at 37°C for 30 minutes with constant shaking, then centrifuged at 2000 x g for 10 minutes and the supernatant was removed. Oxidative stress was induced by adding 0.5 mL of 200 mmol H_2O_2 to all samples except the control group where 0.5 mL of PBS was added, then incubated at 37°C for 30 minutes with constant shaking. For MDA determination, 0.2 mL of each sample was taken and re-suspended in 3 mL of PBS containing 0.5 mmol of glucose. From the new solution, 0.2 mL was taken and 1 mL of 20% trichloroacetic acid (TCA) was added, shaken, centrifuged at 2000 x g, 1 mL of supernatant was taken and 2 mL of 0.67% thiobarbituric acid was added, the samples were placed in a vessel with boiling water and incubated for 20 minutes, at the end of which the samples were immediately cooled on ice to stop the reaction, then the absorbance was determined at 532 nm. The MDA concentration was calculated with the molar extinction coefficient (e = 156 000 M-1 cm-1).

Induction of inflammation with carrageenan 2% and evaluation of the anti-inflammatory effect

The method is based on mechanical rupture of the subcutaneous connective tissue which is achieved by injecting air into the dorsum of the mice resulting in lining structures with many characteristics of the synovial membrane 6 days after the initial injection[24]. Six groups of mice were randomly formed (blank, carrageenan control, dexamethasone

2 mg/kg, ChS70 100 mg/kg, ChS70 250 mg/kg, and ChS70 500 mg/kg). The evaluation of inflammation prevention was performed by determining MDA, NO, total proteins, albumin and leukocytes in exudate, as well as the histological structure of the air sac membrane.

Inflammation was induced by subcutaneous administration of sterile air and 2% carrageenan in the scapular area of the mice of all groups according to Duarte et al[25]. On the first day 6 mL of sterile air was administered subcutaneously in the scapular area, on the third day 3 mL of air was administered additionally, on the sixth day, on an empty stomach, the treatments were administered orally to each group: placebo (solvent of the extracts) was administered to the first and second groups, dexamethasone 2mg/kg to the third group, ChS70 100 mg/kg to the fourth group, ChS70 250 mg/kg to the fifth group and ChS70 500 mg/kg to the sixth group. After 3 hours, the groups treated with ChS70, dexamethasone and carrageenan control were given 1 mL of 2% carrageenan in the air bag and the white group was given 1 mL of saline solution. After 3 hours, the groups treated with ChS70, dexamethasone and carrageenan control were given 1 mL of 2% carrageenan in the air bag and the white group was given 1 mL of saline solution. Twenty-four hours later, mice were euthanized as described by Chow et al. and Huang[26,27] with pentobarbital at a dose of 100 mg/kg intraperitoneally, then the air sac was incised, the exudate was collected in ice-cold test tubes, centrifuged at 3000 x g, the supernatant was used for determination of biochemical parameters and the precipitate for determination of leukocyte number and differentiation. A segment of the air sac tissue was fixed in buffered formalin for histological evaluation.

Albumin content, total protein, number and differentiation of leukocytes

Albumin and total proteins were evaluated in the supernatant with the URIT 810 semi-automated biochemical analyzer. For the number of leukocytes, 0.5 mL of precipitate was taken and diluted with 1.5 mL of saline solution and from this solution the number and differentiation of leukocytes was determined in an automated hematological analyzer FAR LAB 2900 PLUS.

Malondialdehyde content in exudate

The rationale is based on the fact that MDA reacts with thiobarbituric acid (TBA) to form a colored compound. Quantification was performed according to Drapee et al and Buege[28,29]. To 0.3 mL exudate 0.6 mL of 20% trichloroacetic acid was added, shaken for 5 min, centrifuged and 1 mL of supernatant was taken, immediately 0.9 mL of 0.67% thiobarbituric acid diluted in 0.25 N hydrochloric acid was added, then brought to boiling water bath for 30 min, immediately cooled with ice water to stop the reaction. The MDA content was determined in a UV-VIS spectrophotometer at 535 nm. The malondialdehyde concentration was calculated using the extension coefficient (e = 15,600 M-1x cm-1)

Nitric oxide content in exudate

Nitrites in the exudate were determined by the Griess reaction according to Palmer et al[30]. For deproteinization to 0.8 mL of exudate was added 0.6 mL of distilled water, 0.1 mL of 1 M sodium hydroxide (NaOH) and. 1 mL of zinc sulfate ($ZnSO_4$) at 30%, after being subjected to a magnetic stirrer for 5 minutes, it was centrifuged at 3000 x g for 10 minutes. To 1 mL of supernatant an aliquot of zinc metal powder was added and allowed to stand for 1 hour and 45 minutes with constant shaking, then centrifuged at 3000 x g for 10 minutes. To 0.5 mL of supernatant was added 0.25 mL of Griess reagent A and after 5 minutes 0.25 mL of Gries B, finally incubated for 30 minutes at room temperature. The absorbance was determined at 550 nm in a UV-VIS spectrophotometer. The total nitrite concentration was performed on a curve made with sodium nitrite standard.

Histological analysis

Preparation of slides for histopathological evaluation was performed according to Jun-Eun[31], an air sac segment was fixed in 10% (v/v) buffered formalin, dehydrated in ethanol series (99.9%, 80%, and 70%), then embedded in kerosene. The tissue section was 4 µm and stained with hematoxylin and eosin. Observation of the histological structure was performed with a LEICA DM500 microscope at 10x.

Statistical analysis

In the results of the biochemical parameters of exudate, the mean +/- mean standard error (EEM) were determined; in the first stage, homogeneity of variance was determined using the Leve test and then normality with Shapiro Wilk. In the second stage, the ANOVA test was performed to verify the variance, and finally the Tukey test was performed to evaluate the difference between the groups. The correlation between the variables of antioxidant capacity and phenolic compounds was performed with Pearson's test. All analyses were evaluated with a significance level of p<0,01 and p<0,05. The data were processed with Excel and SPSS software.

RESULTS

Taxonomic classification of the species and obtaining hydroalcoholic extracts

The plant species was identified as ***Chuquiraga spinosa*** Less**.** according to certificate No. 242-USM-2019, issued on July 31, 2019 by the Natural History Museum of the Universidad Nacional Mayor de San Marcos. Extracts ChS96, ChS70, ChS50 and ChSAc were obtained according to the flow chart in Figure 1.

Table 2 shows that the ChSAc extract has the highest capacity to scavenge DPPH free radicals. Regarding the inhibition of MDA release, it was observed that the ChS70 extract had greater efficacy without significant difference compared to the results of the ChS50 and ChSAc extracts. In terms of polyphenol content, the ChSAc extract presented a higher amount without significant difference with the ChS70 extract.

When evaluating the correlation between the total polyphenol content with the capacity to reduce DPPH radical and with the inhibition of MDA release, values of -0.909 and -0.781 were obtained, respectively, with a significance level of p<0.001, showing that the polyphenol content influences the capacity to eliminate DPPH and inhibit MDA.

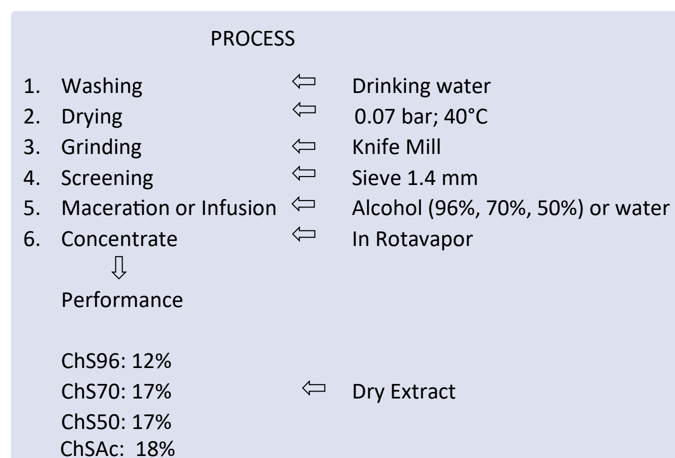


Figure 1: Obtaining and yield of dry extracts of Chuquiraga spinosa Less, ChS96: ethanolic extract 96%; ChS70: ethanolic extract 70%; ChS50: ethanolic extract 50% and ChSAc: aqueous extract.

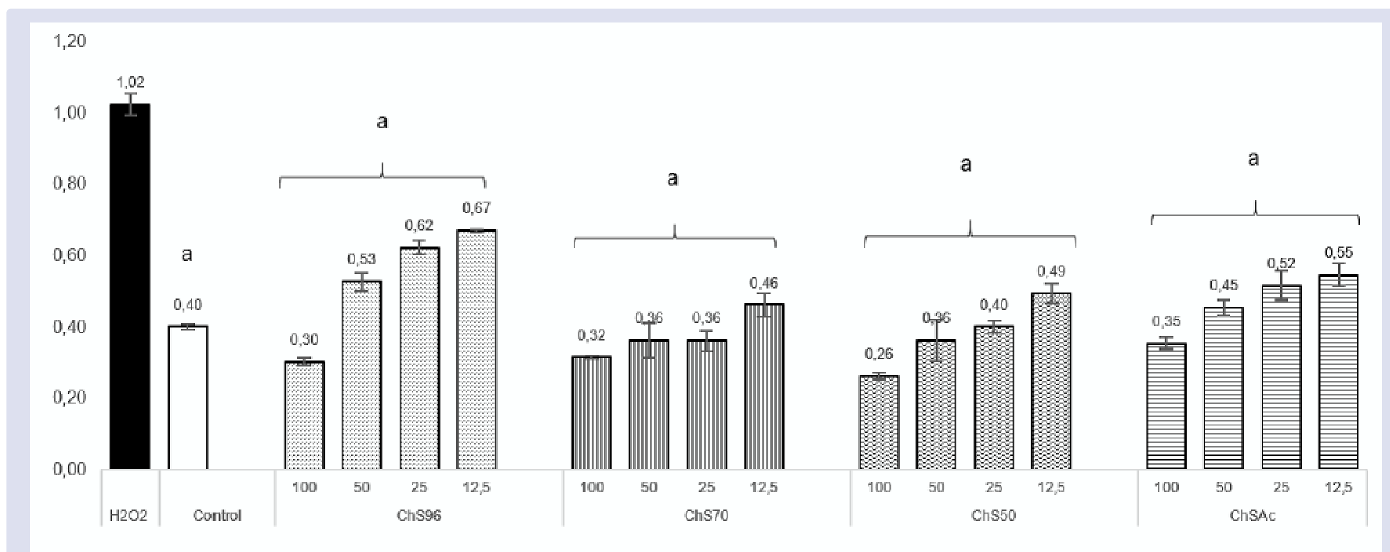


Figure 2: Shows that the ChSAc extract has the highest capacity to scavenge DPPH free radicals. Regarding the inhibition of MDA release, it was observed that the ChS70 extract had greater efficacy without significant difference compared to the results of the ChS50 and ChSAc extracts. In terms of polyphenol content, the ChSAc extract presented a higher amount without significant difference with the ChS70 extract.

Concentration of extracts (µg/mL) versus MDA release (nmol/mL of red blood cells) by H_2O_2-induced oxidative stress in erythrocytes compared with control group and H_2O_2 group, where a: indicates significant difference ($p < 0,01$) when compared with H_2O_2 group. MDA: malondialdehyde, H_2O_2: hydrogen peroxide 200 mmol.

Table 1: Identification of phytochemical constituents of the extracts of Chuquiraga Spinosa Less.

Active metabolites	Reagents	ChS96	ChS70	ChS50	ChSAc
Alkaloids	Mayer	+	+	+	+
	Dragendorff	+	+	+	+
	Bertrand	+	+	+	+
Flavonoids	Shinoda	+	+	+	+
	Aluminum trichloride	+	+	+	+
Terpenes	Sulfuric Vanillin	+	+	+	+
Quinones	Borntranger	-	+	+	+
Phenolic compounds	Ferric trichloride	+	+	+	+
Saponins	Sample + 1% distilled water	-	-	-	-
Tannins	Gelatin/NaCl	-	+	+	+
Carbohydrates	Molish	+	+	+	+
Reducing sugars	Benedict	-	+	+	+
	Feling A and B	-	+	+	+
Triterpenes and steroids	Lieberman - Burchard	+	+	+	+
Sesquiterpene lactones	Baljet A and B	+	+	+	+

(-) = Absence; (+) = Presence.

DISCUSSION

Alkaloids, flavonoids, quinones, phenolic compounds, tannins, triterpenes, steroids, sesquiterpene lactones, amino groups and sugars were identified in the four extracts (Table 1). It was also demonstrated that total polyphenols are related to the reduction of the DPPH radical and the inhibition of lipoperoxidation in erythrocytes, with the 50% and 70% ethanolic extracts showing the greatest antioxidant capacity (Table 2 and Figure 2). This antioxidant capacity is due to the fact that polyphenols donate electrons to free radicals[32] and protect erythrocytes from lipoperoxidation by preventing exposure to reactive oxygen species[33]. In previous studies it was demonstrated that the ethanolic and methanolic extracts have antioxidant capacity[15] as well as that the variation of polarity with the water-methanol mixture increases the antioxidant activity[12], which agrees with the results of the present study carried out with the water-ethanol mixture where the concentrations of the 50% and 70% solvent had greater antioxidant capacity with respect to that of 96%. However, the 70% concentration had a greater capacity to inhibit lipoperoxidation in erythrocytes, so that from this concentration it would be possible to carry out identification and quantification tests of bioactive metabolites, thus establishing quality parameters according to the content of these metabolites.

The ChS70 extract was evaluated in the prevention of carrageenan-induced inflammation in the air sac model in mice. This model is used for studies of various types of inflammation, and has an advantage over other models due to the accessibility of samples for biochemical analysis in the exudate and the air sac membrane for evaluation of inflammatory cells and angiogenesis in histological studies[34]. In this study it was evidenced that, after carrageenan injection in the air sac, the number of total leukocytes, NO level, MDA level, total protein content, albumin content in the exudate, as well as leukocyte infiltration in the air sac membrane, increased significantly in the carrageenan control group.

ChS70 treatments decreased albumin and total protein content in a dose-dependent manner, where the group that received 500 mg/kg

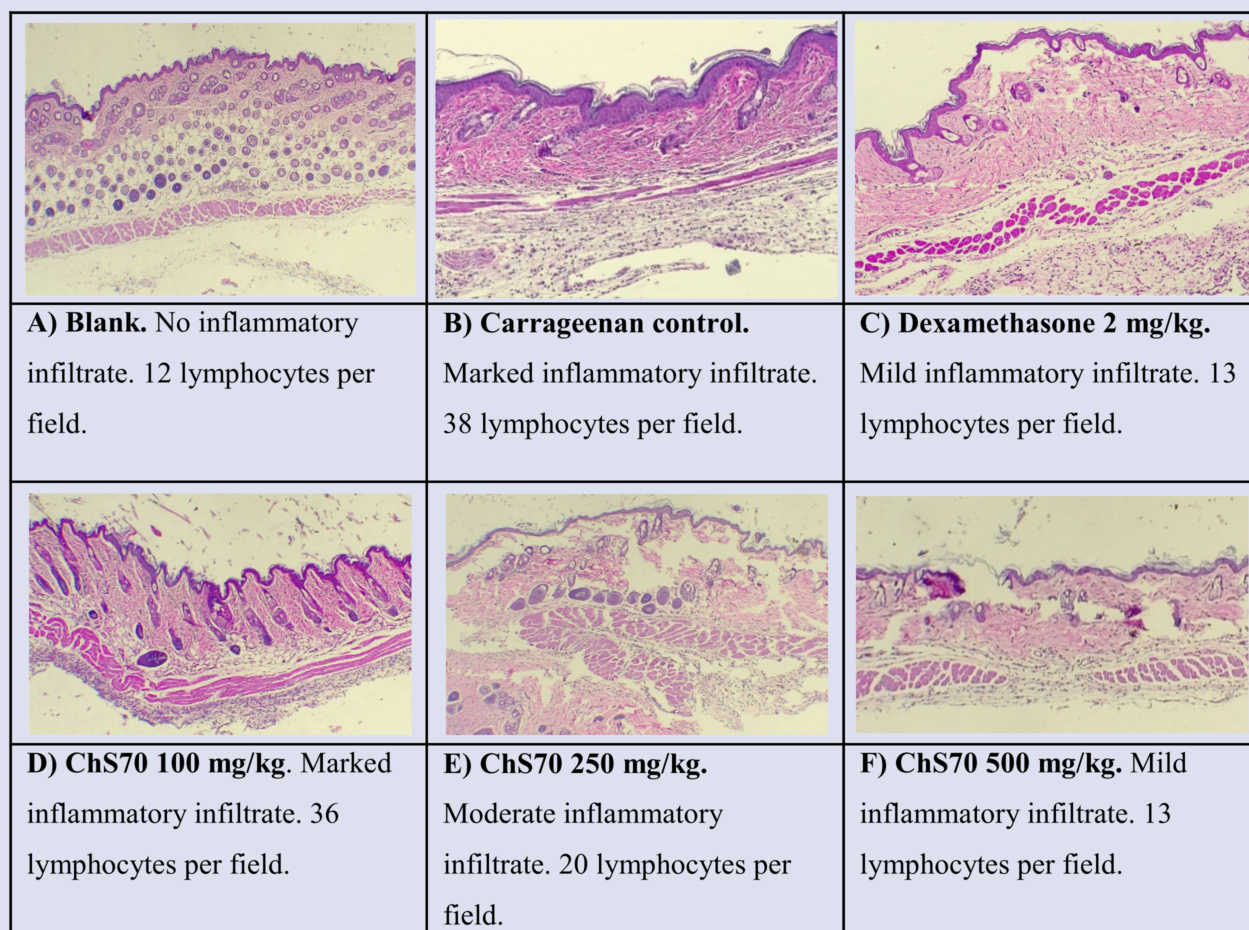


Figure 3: Shows that the air sac membrane is inflamed by leukocyte infiltration in the groups that were administered carrageenan (characteristic of edema) compared to the group that was only administered placebo. Likewise, the groups treated with dexamethasone 2mg/kg, ChS 250 mg/kg and ChS 500 mg/kg showed less leukocyte infiltration in the air sac membrane compared to the control group. In addition, a lower number of lymphocytes per field was evidenced in the treated groups when compared to the control group.

Effect of ChS on the leukocyte infiltrate in the membrane covering the air sac after administration of carrageenan 2%. Magnification 10X and staining with hematoxylin and eosin. PBS: Physiological saline solution.

Table 2: DPPH radical reduction, MDA release inhibition and total polyphenols of Chuquiraga Spinosa Less extract.

Sample	DPPH# reduction	Inhibition of MDA+ release	Total polyphenols++
ChS96	82,23^{a} +/- 0,26	28,80^{a} +/- 1,18	55,79^{a} +/- 2,39
ChS70	68,43^{b} +/- 0,31	16,44^{b} +/- 0,15	120,20^{b} +/- 1,75
ChS50	64,70$^{b, c}$ +/- 0,68	17,59^{b} +/- 0,03	108,44^{c} +/- 2,11
ChSAc	58,99^{c} +/- 2,70	21.83^{b,a} +/- 1.92	125,90^{b} +/- 0,89
AA	2,75^{d} +/- 0,15	-	-

DPPH radical reduction capacity expressed as CI50 (µg/mL); +MDA inhibition capacity expressed as CI50 (nmol/mL); ++Polyphenol content expressed as milligrams of gallic acid (GA) per gram of dry extract. In each column values on average (n=3, + EEM), different letters indicate significant difference (p< 0,01) based on Tukey's multiple comparison test.

Table 3: Effect of ChS70 and dexamethasone on biochemical parameters in exudate after administration of 2% carrageenan in air bag.

Group (n)	Malondialdehyde	Nitric oxide	Albumin	Total protein
	nmol/mL	µmol/L	g/dL	g/dL
Blank	0,20^{a} +/- 0,01	1,02^{a} +/-0,17	0,27^{a}+/-0,02	2,12^{a} +/-0,01
Control (Carrageenan 2%)	0,99 +/- 0,09	31,58 +/-0,89	1,82 +/-0,02	5,30 +/-0,01
Dexamethasone 2mg/kg	0.49^{a,b,c} +/-0.05	3.30^{a,b} +/-0.91	1,23^{a} +/-0,01	3.54^{a,b} +/-0.02
ChS70 100 mg/Kg	0.63^{a,c} +/-0.03	24,40^{a} +/-0,84	1,63^{a}+/-0,02	4,59^{a} +/-0,01
ChS70 250 mg/Kg	0.58^{a,c} +/-0.09	17,19^{a} +/-1,46	1,50^{a} +/-0,02	4,16^{a} +/-0,01
ChS70 500 mg/Kg	0.44^{a,b,c} +/-0.02	5,71^{a,b} +/-0,58	1,07^{a} +/-0,03	3.63^{a,b}+/-0.03

In each column the values are in average (n = 6, + EEM), where a: indicates significant difference (p < 0,01) compared to the control group and b,c: indicates that there is no significant difference (p > 0,01).

Table 4: Effect of ChS70 and dexamethasone on leukocytes in exudate after administration of 2% carrageenan in air bag.

Groups (n)	Leukocytes x 10^3/mm^3	Leukocyte differentiation		
		Polymorphonuclear x 10^3/mm^3	Lymphocytes x 10^3/mm^3	Monocytes x 10^3/mm^3
Blank	3,36[a] +/-0,30	0,42[a] +/-0,04	2,37[a] +/-0,22	0,57[a] +/-0,06
Control (Carrageenan 2%)	24,07 +/-0,81	1,59 +/-0,13	20,54 +/-0,74	3,75 +/-0,28
Dexamethasone 2 mg/kg	11,57[a]+/- 0,21	0,63[a] +/-0,05	9,36[a] +/-0,08	1.58[a,b] +/-0.14
ChS70 100 mg/Kg	22,46 +/-0,40	1,31[b] +/-0,02	17,11[a,c] +/-0,55	2,30[a,b] +/-0,04
ChS70 250 mg/Kg	18.48[a,b] +/-0.21	1,18[a,b] +/-0,03	15,20[a,b,c] +/-0,15	2,23[a,b] +/-0,10
ChS70 500 mg/Kg	16,26[a,b] +/-0,30	0.99[a,b] +/-0.03	13,32[a,b] +/-0,25	1.76[a,b] +/-0.11

In each column the values are in average (n = 6, +/-EEM), where a: indicates significant difference ($p < 0,01$) compared to the control group and b, c: indicates that there is no significant difference ($p > 0,01$).

had a greater effect. Carrageenan-induced inflammation is associated with protein leakage into the air sac, mediated by inflammatory factors such as bradykinin, serotonin, histamine, prostaglandins[35]. In the inflammatory process at the site of aggression, an initial vasodilatation is observed, increasing vascular permeability, cell activation-adhesion and hypercoagulability. Vasodilatation and increased microvascular permeability at the site of inflammation increase the local availability of nutrients and oxygen, producing heat, swelling and tissue edema[36].

The groups treated with dexamethasone, ChS70 500, 250 and 100 mg/kg reduced the migration of leukocytes in the exudate by 52%, 33%, 23% and 7% respectively, showing a greater decrease with dexamethasone followed by ChS70 500 mg/kg. In the presence of exogenous inflammatory agents such as carrageenan, in this case leukocytes are the first line of defense and when phagocytizing they release reactive oxygen species (ROS) such as hydroxyl and hypochlorous acid[37]. Excess ROS can damage leukocytes but they are protected by enzymes such as SOD, CAT[38], however when damage persists cytotokines (IL-1, TNF-α) and lipopolysaccharides provoke greater migration of leukocytes which will favor the accumulation of phospholipase A_2 (PLA2) in inflammatory fluids and plasma[39] releasing arachidonic acid which by the action of cyclooxygenase (CO) and lipoxygenase (LO) forms inflammatory mediators such as prostaglandins, thromboxanes and leukotrienes[40]. In the present study, a dose-dependent effect of ChS70 extract was evidenced by decreasing leukocytes, polymorphonuclear cells, lymphocytes, and monocytes. Segmented neutrophils have an important role in the immunopathogenesis of inflammation as they can not only generate ROS but also chemoactive agents (LTB4 and 5-HETE) favoring a greater accumulation of segmented neutrophils in inflammatory exudates as well as synovial fluid[41]. As for lymphocytes, these fulfill specific autoimmune function, where T lymphocytes favor greater proliferation of the same T lymphocytes, they also activate B lymphocytes to form specific antibodies, cytotoxic lymphocytes directly eliminate external agents and natural killer lymphocytes promote apoptosis. Monocytes/macrophages, polymorphonuclear leukocytes and endothelial cells are involved in the inflammatory response, when activated they aggregate and leak into the tissue producing a respiratory burst, increasing oxygen use, cytokine production, generation of ROS and other inflammatory mediators[42].

The NO in the carrageenan control group (31.58 μmol/L) was 31 times the value of the target group (1.02 μmol/L). The group treated with dexamethasone and the groups treated with ChS70 extract at doses of 500 mg/kg, 250 mg/kg and 100 mg/kg decreased by 90%, 82%, 46% and 23%, respectively. The mechanism by which NO inhibition is anti-inflammatory is not well defined, however, it has been demonstrated that nitric oxide when interacting with superoxide induces the formation of peroxynitrite causing endothelial dysfunction and lipoperoxidation releasing arachidonic acid[43,44] it also stimulates the expression of the COX-2 enzyme producing inflammatory prostaglandins such as PG2[42]. It is known that the products that reduce NO production by more than 65% could be related to the inhibition of NF-kB expression[45].

The MDA content in the exudate of the control group had a significant increase, while the groups treated with dexamethasone and ChS70 at doses of 500, 250 and 100 mg/kg decreased by 51%, 56%, 41% and 36%, respectively. MDA is a biomarker of lipoperoxidation which is an oxidative degenerative process of membrane lipids of the endoplasmic reticulum. These lipids are rich in polyunsaturated fatty acids[46]. Likewise, MDA can react with DNA bases forming mutagenic and carcinogenic etheno-DNA adducts[47]. Increased lipoperoxidation by carrageenan injection into the air sac is mediated by NO production[48].

Dexamethasone inhibits iNOS activity in vitro and in vivo, without affecting constitutive nitric oxide (cNOS)[49,50], blocking the protein expression of iNOS and therefore suppressing NO release caused by carrageenan, it also reduces the migration of neutrophils, macrophages, as well as TNF-α and MDA levels[51]. The ChS70 500mg extract would have the capacity to modify and prevent inflammatory processes considering that part of its chemical components are glycosylated flavonoids from the flavonol base Kaempferol, quercetin and isorhamnetin[11] and that these flavonols inhibit the expression of the iNOS protein as well as the activation of NF-kB and STAT-1 which are transcription factors for iNOS, decreasing NO production[13] similarly, quercetin inhibits the release of β-glucuronidase from polymorphonuclears as well as PLA2 activation decreasing arachidonic acid release[52] and Kaempferol modulates cyclooxygenase expression by inhibiting iNOS preventing the formation of inflammatory eicosanoids[42]. Considering that dexamethasone had the same profile as ChS70 on the number of leukocytes, NO, and MDA in exudate, although the effect was greater, it is likely that both have a common pathway of action in the inflamed area. Products that reduce NO production are promising for the prevention of inflammatory processes in chronic diseases[53,54].

The anti-inflammatory effect was also evaluated in histological changes of the air sac membrane, migration of leukocyte cells and number of lymphocytes per field. In the carrageenan control group, thickened membrane thickness, increased presence of leukocytes, increased number of lymphocytes, prominent granule production, granuloma formation in the epidermal-dermal layer and keratinization of the epidermal layer were observed. In the groups treated with dexamethasone and ChS70, decreased signs of inflammation were observed in a dose-dependent manner, where the groups treated with ChS70 500 mg/kg and dexamethasone had a greater effect. The air sac model is usually used to evaluate acute and chronic inflammation, due to the easy integration and interpretation of the results[55], likewise, it is one of the most useful models to evaluate the anti-arthritic effect due to the fact that the membrane of the air sac is similar in its cellular structure to the synovial membrane, allowing the evaluation of the cellular components of the synovial membrane[17,24].

The results of the present investigation complement the studies carried out on the anti-inflammatory effect of the aerial parts of *Chuquiraga spinosa*, where it was evidenced that the methanolic extract at 50% had an anti-inflammatory effect in a model of subplantar edema and ear in mice with a decrease in inflammation of 52,5% at a dose of 500 mg/kg and 88.07% at a dose of 2.5 mg/ear respectively[12], as well as the

chloroformic extract decreased inflammation by 39.1% in a model of subplantar edema in rats at a dose of 300 mg/kg[16] The subplantar and ear edema models allow a general anti-inflammatory evaluation, while the air sac model with carrageenan facilitates the evaluation of changes in biochemical, immunological and histological parameters. Therefore, the evaluated extract ChS70 at a dose of 500 mg/kg would have an anti-inflammatory effect by reducing MDA, NO and leukocyte migration, and could be evaluated in more specific inflammatory processes according to traditional use in order to demonstrate efficacy, without neglecting safety studies.

LIMITATIONS

- It was demonstrated that the antioxidant capacity is related to the content of polyphenols in hydroalcoholic extract, however, it is also necessary to quantify the totality of bioactive components.
- Evaluation of the effect on the inflammation process as a function of iNOS expression, NFkB expression and cytokine determination.

CONCLUSIONS

Ethanolic extracts (96%, 70% and 50%) and aqueous infusion of the aerial parts of *Chuquiraga spinosa* Less, have antioxidant capacity against the DPPH radical and prevent H_2O_2-induced lipoperoxidation in erythrocytes in a dose-dependent manner and these activities are correlated with the content of total polyphenols.

Administration of 70% ethanolic extract of *Chuquiraga spinosa* Less (ChS70) parts areas at doses of 100, 250 and 500 mg/kg in mice with carrageenan-induced inflammation in the air sac prevented the release of nitric oxide, malondialdehyde, total protein migration, albumin migration and leukocyte infiltration in the exudate, as well as protection of histological changes in the air sac membrane, with the 500 mg/kg dose showing the greatest effect.

ACKNOWLEDGMENTS

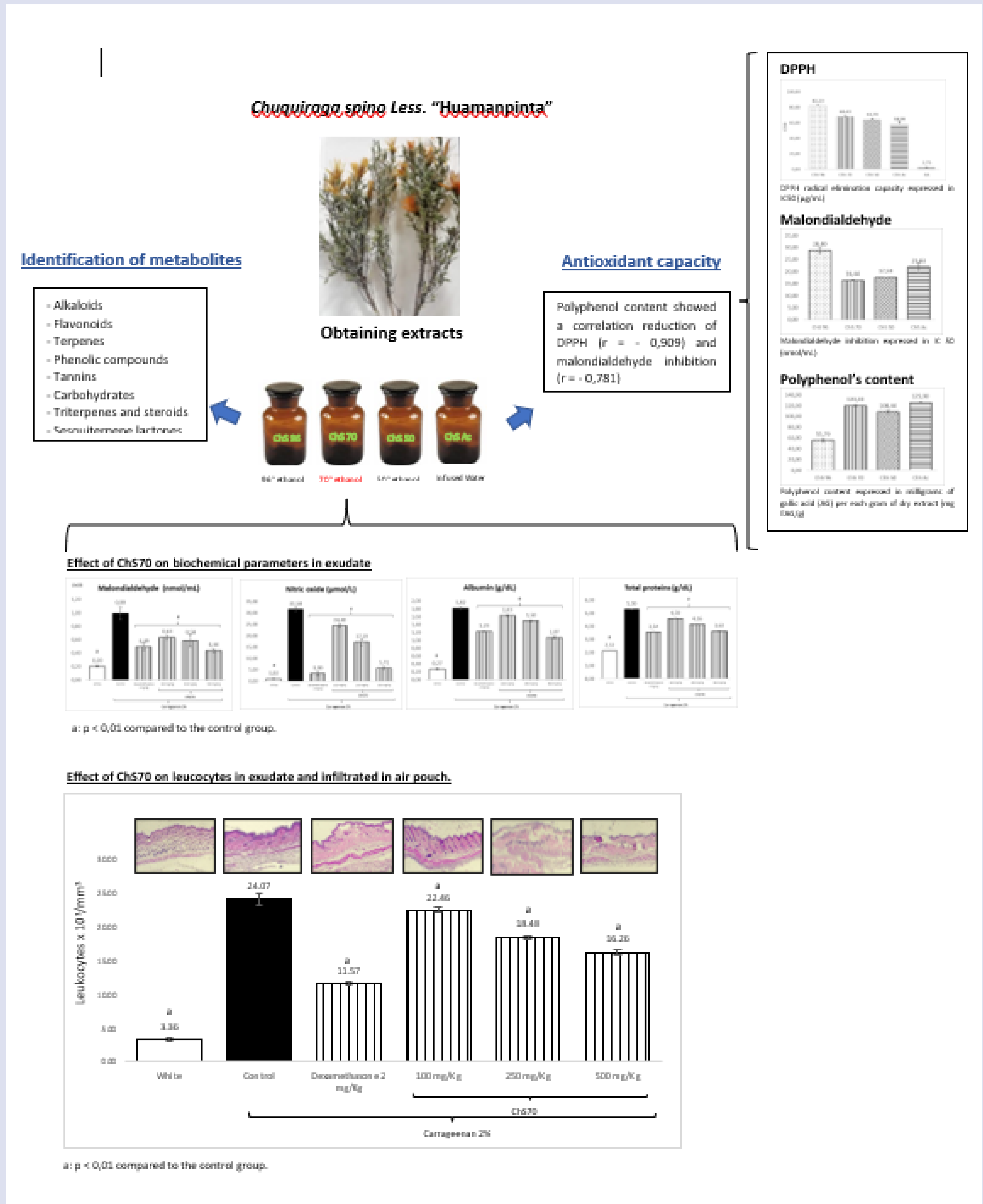
We are grateful for the support of the Research Unit of the Faculty of Medicine of the UNMSM for allowing the execution of the experimental part with animals in the biotherium of the Faculty; we are also grateful to the Direction of the Department of Dynamic Sciences for allowing the execution of the biochemical and phytochemical analysis part of the research in the Pharmacology laboratory.

REFERENCES

1. Ghezzi P, Jaquet V, Marcucci F, Schmidt HHHW. The oxidative stress theory of disease: levels of evidence and epistemological aspects. Br J Pharmacol. 2017;174(12):1784-96.
2. Ayala A, Muñoz M, Argüelles S. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal Antonio. Oxid Med Cell Longev [Internet]. 2014 [cited 2018 Dec 28];2014:1-31. Available from: http://dx.doi.org/10.1155/2014/360438
3. Clemens MR, Waller HD. Lipid peroxidation in erythrocytes. Chem Phys Lipids. 1987;45(2-4):251-68.
4. Halliwell B. The Role of Oxygen Radicals in Humana Disease, with Particular Reference to the Vascular System. Haemostasis. 1993;23(1):118-26.
5. Comporti M. Three models of free radical-induced cell injury. Chem Biol Interact [Internet]. 1989 Jan 1 [cited 2018 Oct 8];72(1-2):1-56. Available from: https://doi.org/10.1016/0009-2797(89)90016-1
6. Mehta JL, Rasouli N, Sinha AK, Molavi B. Oxidative stress in diabetes: A mechanistic overview of its effects on atherogenesis and myocardial dysfunction. Int J Biochem Cell Biol [Internet]. 2006 May [cited 2018 Oct 8];38(5-6):794-803. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16442834
7. Halliwell B, Gutteridge JM. The importance of free radicals and catalytic metal ions in human diseases. Mol Aspects Med [Internet]. 1985 [cited 2018 Oct 8];8(2):89-193. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3908871
8. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact [Internet]. 2006 Mar 10 [cited 2018 Oct 8];160(1):1-40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16430879
9. Wang W, Bergh A, Damber JE. Chronic inflammation in benign prostate hyperplasia is associated with focal upregulation of cyclooxygenase-2, Bcl-2, and cell proliferation in the glandular epithelium. Prostate. 2004;61(1):60-72.
10. Mostacero J, Mejía F, Gamarra O. Phanerogams of Peru: taxonomy, utility and ecogeography [Internet]. CONCYTEC, editor. Trujillo; 2009 [cited 2018 Nov 18]. Available from: https://books.google.com.pe/books/about/Fanerógamas_del_Perú.html?hl=es&id=rrBqtwAACAAJ&redir_esc=y
11. Landa A, Casado R, Calvo MI. Identification and quantification of flavonoids from Chuquiraga spinosa (Asteraceae). Nat Prod Commun [Internet]. 2009 Oct [cited 2018 Mar 12];4(10):1353-5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19911570
12. Casado R, Landa A, Calvo J, García-Mina JM, Marston A, Hostettmann K, et al. Anti-inflammatory, antioxidant and antifungal activity of Chuquiraga spinosa. Pharm Biol. 2011;49(6):620-6.
13. Hämäläinen M, Nieminen R, Vuorela P, Heinonen M, Moilanen E. Anti-inflammatory effects of flavonoids: Genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-κB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-κB activation along with their inhibitory effect on i. Mediators Inflamm. 2007;2007:1-10.
14. Herrera O, Tinco J, Franco C, Chumpitaz V, Castro W, Pari B, et al. Antioxidant activity and cytotoxic profile of Chuquiraga spinosa Lessing on human tumor cell lines: A promissory plant from Peruvian flora. Asian Pac J Trop Dis [Internet]. 2017 [cited 2018 Feb 23];7(5):304-8. Available from: https://doi.org/10.12980/apjtd.7.2017D6-436
15. Arroyo J, Herrera O, Chavez R, Anampa A, Chumpitaz V, Enciso E. Protective effect of Chuquiraga spinosa extract on N-methyl-nitrosourea (NMU) induced prostate cancer in rats. Prostate Int [Internet]. 2017 [cited 2018 Feb 17];5:47-52. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5448729/pdf/main.pdf
16. Ramírez E, Bonilla P, Suarez S, Choquesillo F, Castro A. Antioxidant, antiinflammatory and immunomodulatory activity of the chloroform extract from the leaves of Chuquiraga lessing "HUAMANPINTA." Cienc Invest [Internet]. 2015 Mar 20 [cited 2018 Apr 13];17(1):37-42. Available from: http://revistasinvestigacion.unmsm.edu.pe/index.php/farma/article/view/11116
17. Edwards JCW, Sedgwick AD, Willoughby DA. The formation of a structure with the features of synovial lining by subcutaneous injection of air: An in vivo tissue culture system. J Pathol. 1981;134:147-56.
18. National-Research-Council. Guide for the Care and Use of Laboratory Animals [Internet]. National Academies Press (US); 2011 [cited 2018 Jul 28]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21595115
19. Farmacopea-Estados-Unidos-De-América. USP 37 - NF 32 The United States Pharmacopeia and National Formulary 2014. Metodo <565> Pag. 321. 2014. 321-324 p.
20. Lock O. Phytochemical Research. Methods in the study of Natural Products. 3rd ed. Lima: PUCP; 2016.
21. Prieto JM. Procedure : Preparation of DPPH Radical , and antioxidant scavenging assay. DPPH Microplate Protocol. 2012.
22. Quispe-Mendoza L, Betalleluz-Pallardel I, Vargas-Delgado L, Velezmoro-Sánchez C. Stability of cooked pressed yellow potato (Solanum goniocalyx) in frozen storage. Sci Agropecu. 2018;9(1):113-21.

23. Stocks J, Dormandy TL. The Autoxidation of Human Red Cell Lipids Induced by Hydrogen Peroxide. Br J Haematol. 1971;20(1):95-111.
24. Sedgwick AD, Sin YM, Edwards JCW, Willoughby DA. Increased inflammatory reactivity in newly formed lining tissue. J Pathol. 1983;141:483-95.
25. Duarte DB, Vasko MR, Fehrenbacher JC. Models of inflammation: Carrageenan air pouch. Curr Protoc Pharmacol. 2012;(5):1-12.
26. Chow LH, Chen YH, Wu WC, Chang EP, Huang EYK. Sex difference in oxytocin-induced anti-hyperalgesia at the spinal level in rats with intraplantar carrageenan-induced inflammation. PLoS One. 2016 Sep;11(9):1-15.
27. Romero-Fernandez W, Batista-Castro Z, De Lucca M, Ruano A, García-Barceló M, Rivera-Cervantes M, et al. The 1, 2, 3 of experimentation with laboratory animals. Rev Peru Med Exp Salud Publica. 2016;33(2):288-99.
28. Draper HH, Squires EJ, Mahmoodi H, Wu J, Agarwal S, Hadley M. A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. Free Radic Biol Med. 1993;15(4):353-63.
29. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978 Jan 1;52:302-10.
30. Palmer R, Ferrige A, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature [Internet]. 1987 Jun 11 [cited 2018 Feb 3];327(6122):524-6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3495737
31. Kim JE, Park KM, Lee SY, Seo JH, Yoon IS, Bae CS, et al. Anti-inflammatory effect of Allium hookeri on carrageenan-induced air pouch mouse model. PLoS One. 2017 Dec 1;12(12):1-11.
32. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, et al. Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. Int J Mol Sci. 2017 Jan 5;18(96):1-32.
33. Fatima M, Kesharwani RK, Misra K, Rizvi SI. Protective effect of theaflavin on erythrocytes subjected to in vitro oxidative stress. Biochem Res Int [Internet]. 2013 [cited 2018 Oct 2];2013:649759. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24455262
34. Eteraf-Oskouei T, Akbarzadeh-Atashkhosrow A, Maghsudi M, Najafi M. The Impact of Gender on the Inflammatory Parameters and Angiogenesis in the Rat Air Pouch Model of Inflammation. Res Pharm Sci. 2009;12(5):364-72.
35. Posadas I, Bucci M, Roviezzo F, Rossi A, Parente L, Sautebin L, et al. Carrageenan-induced mouse paw oedema is biphasic, age-weight dependent and displays differential nitric oxide cyclooxygenase-2 expression. Br J Pharmacol. 2004;142(2):331-8.
36. García de lorenzo y mateos A, López martínez J, Ssánchez castilla M. Systemic inflammatory response: Pathophysiology and mediators. Med Intensiva. 2000;24(8):353-60.
37. Yasui K, Baba A. Therapeutic potential of superoxide dismutase (SOD) for resolution of inflammation. Vol. 55, Inflammation Research. 2006. p. 359-63.
38. Salin ML, McCord JM. Free radicals and inflammation. Protection of phagocytosing leukocytes by superoxide dismutase. J Clin Invest. 1975;56(5):1319-23.
39. Valdés Y, Bilbao Mi, León J, Francisco M. Origin and Importance of Phospholipase A2 Secretion. Rev Cuba Farm. 2002;36(2):121-8.
40. Gonzalez H, Smith D, Turner R. Arachidonic metabolism and Phospholipase A2 activityin patients with rheumatoid arthritis. Acta méd colomb. 1990;15(1):21-4.
41. Klickstein LB, Shapleigh C, Goetzl EJ. Lipoxygenation of arachidonic acid as a source of polymorphonuclear leukocyte chemotactic factors in synovial fluid and tissue in rheumatoid arthritis and spondyloarthritis. J Clin Invest [Internet]. 1980 [cited 2020 Nov 26];66(5):1166-70. Available from: /pmc/articles/PMC371556/?report=abstract
42. Mahat MYA, Kulkarni NM, Vishwakarma SL, Khan FR, Thippeswamy BS, Hebballi V, et al. Modulation of the cyclooxygenase pathway via inhibition of nitric oxide production contributes to the anti-inflammatory activity of kaempferol. Eur J Pharmacol [Internet]. 2010 [cited 2020 Jun 19];642(1-3):169-76. Available from: http://dx.doi.org/10.1016/j.ejphar.2010.05.062
43. Ceriello A simultaneous control of hyperglycemia and G-1 infusion normalize endothelial function in type 1 diabetes, De Nigris V, Pujadas G, La Sala L, Bonfigli AR, Testa R, et al. The simultaneous control of hyperglycemia and GLP-1 infusion normalize endothelial function in type 1 diabetes. Diabetes Res Clin Pract [Internet]. 2016;114:64-8. Available from: http://dx.doi.org/10.1016/j.diabres.2016.01.019
44. Salvemini D, Settle SL, Masferrer JL, Seibert K, Currie MG, Needleman P. Regulation of prostaglandin production by nitric oxide; an in vivo analysis. Br J Pharmacol. 1995;114(6):1171-8.
45. Cordeiro NM, Freitas RHCN, Fraga CAM, Fernandes PD. Discovery of novel orally active tetrahydro-naphthyl-N-acylhydrazones with in vivo anti-TNF-α effect and remarkable anti-inflammatory properties. PLoS One. 2016;11(5):1-17.
46. Onasanwo S, Oyebanjo O, Ajayi A, Olubori M. Anti-nociceptive and anti- inflammatory potentials of Vernonia amygdalina leaf extract via reductions of leucocyte migration and lipid peroxidation. J Intercult Ethnopharmacol. 2017 Apr 18;6(2):192-8.
47. Peccerella T, Arslic-Schmitt T, Mueller S, Linhart KB, Seth D, Bartsch H, et al. Chronic ethanol consumption and generation of Etheno-DNA adducts in cancer-prone tissues. Adv Exp Med Biol. 2018;1032:81-92.
48. Tanas S, Odabasoglu F, Halici Z, Cakir A, Aygun H, Ali A, et al. Evaluation of anti-inflammatory and antioxidant activities of peltigera rufescens lichen species in acute and chronic inflammation models. J Nat Med. 2010;64(1):42-9.
49. Radomski MW, Palmer RMJ, Moncada S. Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. Proc Natl Acad Sci U S A. 1990;87(24):10043-7.
50. Moncada, S. & Higgs A. L-Arginine-NO pathway. N Engl J Med. 1993;329:2002-12.
51. Abdel Kawy HS. Montelukast versus Dexamethasone Treatment in a Guinea Pig Model of Chronic Pulmonary Neutrophilic Inflammation. COPD J Chronic Obstr Pulm Dis. 2016;13(4):455-63.
52. Lee T-P, Matteliano M, Middleton E. Efect of quercetin on human polymorphonuclear leukocype lysosomal enzyme release and phospholipid metabolism. Life Sci [Internet]. 1982 [cited 2020 Jun 16];31(24):2765-74. Available from: https://doi.org/10.1016/0024-3205(82)90723-8
53. Jia-Li L, Wu JZ, Liu YH, Zhang ZB, Wu QD, Chen H Bin, et al. Patchoulene Epoxide Isolated from Patchouli Oil Suppresses Acute Inflammation through Inhibition of NF-κB and Downregulation of COX-2/iNOS. Mediators Inflamm. 2017;2017:1-14.
54. Alghaithy AA, El-Beshbishy HA, Abdelnaim AB, Nagy AA, Abdel-Sattar EM. Anti-inflammatory effects of the chloroform extract of Pulicaria guestii ameliorated the neutrophil infiltration and nitric oxide generation in rats. Toxicol Ind Health. 2011;27(10):899-910.
55. Sedgwick AD, Moore AR, Al-Duaij AY, Edwards JC, Willoughby DA. The immune response to pertussis in the 6-day air pouch: A model of chronic synovitis. Br J Exp Pathol. 1985;66(4):455-64.

GRAPHICAL ABSTRACT



ABOUT AUTHORS

Mg. Hugo Jesús Justil-Guerrero
He is a professor and researcher in the Faculty of Medicine, Universidad Nacional Mayor de San Marcos, Lima, Peru. His expertise is the design and execution of experimental research for the evaluation of products with effects on anti-inflammatory processes through the use of in vitro and in vivo models with experimental animals.

Dr. Jorge Luis Arroyo-Acevedo
He is a professor and researcher in the Faculty of Medicine, Universidad Nacional Mayor de San Marcos, Lima, Peru. His expertise is Natural products with anticancer properties, and the use of animal models to discover new potential drugs against different types of cancer.

Dr. Juan Pedro Rojas-Armas
Principal professor of the Pharmacology Section of the Faculty of Medicine, Universidad Nacional Mayor de San Marcos. Researcher of pharmacological and toxicological effects of medicinal plants.

Mg. Miriam Palomino-Pacheco
She is a professor and researcher at the Faculty of Medicine, Universidad Nacional Mayor de San Marcos. Her expertise is in natural products and food.

MSc. Magaly Villena-Tejada
Senior professor of the Academic Department of Pharmacy, Faculty of Health Sciences, Universidad Nacional de San Antonio Abad del Cusco, Peru. Master in Sciences, mention in Chemistry, specialty Natural Products and Doctorate studies in Health Sciences. Researcher in the area of Medicinal Plants and Natural Products, as well as in Complementary and Alternative Medicine.

Dr. Wilmer Atilio Segura Vílchez
Surgeon, graduated from Universidad Nacional Federico Villarreal. Specialist in Pathological Anatomy, Universidad Nacional Federico Villarreal. Lima-Peru. Forensic Physician of the Public Ministry, Lima-Peru. Master in Public Health. Medical Auditor. Professor of Pathological Anatomy, Universidad Privada San Juan Bautista.

Cite this article: Justil-Guerrero HJ, Arroyo-Acevedo JL, Rojas-Armas JP, Palomino-Pacheco M, Villena-Tejada M, Segura-Vilchez W. Antioxidant Capacity of *Chuquiraga Spinosa* Less. "Huamanpinta" and Prevention of Carrageenan-Induced Inflammation in Mice. Pharmacogn J. 2021;13(5): 1287-1296.