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Antioxidant activity and hepatoprotector effect of Spilanthes leiocarpa DC in rats with induced liver injury

Abstract

The present work studied the phytochemical profile of an aqueous extract of Spilanthes leiocarpa DC. (SLDC) and its antioxidant activity was determined by different methods (FRAP, DPPH and ABTS). The hepatoprotective effect was also evaluated in rats with hepatic damage induced with paracetamol (2 mg / Kg), providing different concentrations of SLDC aqueous extract (100, 200 and 400 mg / Kg) and comparing the results with a commercial hepatoprotective drug, Silymarin. The results of phytochemical screening determined the presence of flavonoids, other secondary metabolites such as triterpenes, steroids, alkaloids and compounds with free amino groups. The aqueous extract of SLDC showed values of antioxidant activity similar to those of the commercial drug Silymarin in the three methods tested. In the tests in rats with hepatic damage induced with acetaminophen, it was observed that, after the ingestion of SLDC, the levels of the enzymes transaminase aspartate aminotransferase (AST) and alanine aminotransferase (ALT) recovered basal values when treated at 400 mg / Kg (69.83±9.44 and 52.43±9.52, for AST and ALT respectively), an effect similar to that observed by treatment with 100 mg / kg of silymarin (72.24±4.22 and 47.18±6.87).

Keywords: spilanthes leiocarpa DC, antioxidant activity, hepatoprotective

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Introduction

The liver, is a vital organ in the human body, it is the main responsible for the metabolism of carbohydrates, lipids, proteins and detoxification of xenobiotics and drugs. Therefore, the liver is subject to injury due to chronic exposure to drugs, environmental toxic substances, autoimmune diseases, drugs, and other xenobiotics.[1,2] These pathological conditions and the metabolic and vascularization characteristics of this organ make it more vulnerable. Paracetamol, also known as acetaminophen, is widely used as an antipyretic and pain reliever.[3] ecent studies indicate that a paracetamol overdose is the leading cause of acute liver failure in adults in the United States.[4] In adults, paracetamol is involved in 50% of cases of acute liver failure and, in children, in 13% of cases.[5] Paracetamol is mainly metabolized in the liver and is not toxic in adequate doses. However, either accidental or deliberate overdose can lead to hepatotoxicity caused by the reactive metabolite N-acetyl-p-aminobenzoquinoneimine (NAPQI), which causes cellular oxidative stress.[6,7] In June 2009, the Food and Drug Administration (FDA),[8] through an advisory committee, recommended new restrictions that must be in place to protect people from the potential toxic effects of paracetamol. So far no treatment has successfully prevented the progression of liver disease.

The body has antioxidant defense systems that act by preventing the formation of free radicals, blocking their spread or interacting directly with them. This system is made up of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, uric acid, proteins, glucose, sulfhydryl groups, among others. Also, it is possible that natural or synthetic substances with antioxidant capacity are ingested with the diet, such as flavonoids, polyphenols, β-carotene, vitamin E, vitamin C, among others.[9,10] The use of medicinal plants for curative purposes is a practice that has been used for many decades. They have many antioxidant compounds, mainly polyphenols and flavonoids, which have high antioxidant activity.[11] In this sense, the indiscriminate use of paracetamol in our population is a growing critical problem, with the corresponding aforementioned liver damage, so it is essential to look for an alternative.The Spilanthes leiocarpa DC species, from the Asteraceae family, is traditionally used, in parts or in its entirety, in liver diseases, diabetes, and in dental pain due to its anesthetic power,[12] in addition to other properties such as antiscorbutic;[13] However, there is no evidence from scientific studies on its hepatoprotective action, which is why the present study was carried out, from an aqueous extract taking into account its traditional use.

Experimental part

Biological material

Dried leaves of Spilanthes leiocarpa DC. "Flemadera".

- Male Holtzman strain albino rats weighing 180 and 230 g.

Reagents and solvents

- 6-hydroxy-2,5,7,8-tetramethylchrome-2-carboxylic acid 97% (Trolox), 2,4,6 tripyridyltriazine (TPTZ), 2,2'-azino-di-(3-ethylbenzothiazoline-6- sulfonatodiamonium) (ABTS), SigMA; 2,2-diphenyl-1-picrylhydrazil (DPPH) Sigma; methanol, acetic acid, sodium acetate, hydrochloric acid, potassium persulfate, ferric trichloride, Merck; Wiener Lab Enzymatic Aminotrase Set; ultra pure water.

Medicines:

- Paracetamol (APAP), Silymarin (SIL).

Equipment

Reflux equipment, Sartorius analytical balance, Rotavapor brand HEIDOLPH model LABOROTA 4000, with thermostatic water bath

B-480 and vacuum pumps B-270, Unico 2100 UV spectrophotometer, PLC-012 Universal Centrifuge, Gemmy industrial digital water bath 22 L. ycw-010e., Branson 5510 Sonicator, Millipore Direct-QTM 3 Ultra Pure Water Production System, ® 40 (22 µm), Magnetic Stirrer and Digital Heater.

Methodology

Collection, selection, drying and conservation of the sample under study

Spilanthes leiocarpa DC. "Flemadera" was collected in the town of Casablanca, district of Santiago, province and department of Ica at 378 masl in February 2014. A sample portion was sent to the Natural History Museum of the Universidad Nacional Mayor de San Marcos for identification. Subsequently, the entire SLDC plants were selected, manually separating the deteriorated, stained ones and those with signs of attack by insects and / or fungi; then they were subjected to dryness under shade, spreading in thin layers, on a clean surface for a period of 15 days in the natural products chemistry laboratory of the Faculty of Pharmacy and Biochemistry of the San Luis Gonzaga National University of Ica (UNICA), obtaining approximately 500 g of vegetable sample.

Obtaining the aqueous extract

It was carried out by the reflux method, in a 2000 mL flask 160 g of the whole dried plant and 1600 mL of distilled water were placed. The extraction process was carried out for 4 hours. Subsequently, the extract was concentrated to dryness in a rotary evaporator under reduced pressure at a temperature of 40 °C, obtaining 40 g of dark brown dry extract. This extract was used for antioxidant and hepatoprotective activity.

Phytochemical Screening

From the aqueous extract of Spilanthes leiocarpa DC., They obtained 5 fractions with solvents of varying polarities following a phytochemical screening recommended by Lock[14]; In said fractions, a series of reactions was carried out to determine the presence of functional groups and / or secondary metabolites.

Evaluation of antioxidant activity

The antioxidant activity of the extract was evaluated by three different methods. The results are expressed in internationally accepted units as equivalent antioxidant capacity in mM of Trolox, all determinations being carried out in triplicate.

Preparation of the SLDC extract: A 30mg / mL stock solution was prepared from the dry extract, in 50% methanol, then dilutions were prepared with concentration ranges 15; 7.5; 3.75; and 1,825 mg / mL respectively. These same dilutions were used in all methods; Likewise, silymarin drug dilutions are prepared at the same concentrations.

Iron Reduction Antioxidant Power Method (FRAP)

The procedure followed has been described by Benzie and Strain (1999) with slight modifications. The working reagent was prepared, consisting of a mixture of 300 mM acetate buffer (pH=3.6), 10 mM TPTZ in 40 mM HCl and 20 mM ferric trichloride (FeCl3. 6H2O) in a ratio of 10: 1: 1 (v: v: v), once prepared, 3 mL of this reagent were added in a cuvette, and the absorbance was measured at 593 nm. Subsequently, 100 µL of a dilution of the aqueous SLDC extract was added, and it was vortexed for 30 seconds. After 6 minutes of incubation at room temperature, the absorbance reading was taken again at 593 nm, from which the value of the blank was subtracted15. The samples were tested in triplicate.

Inhibition method against the free radical 2,2-Diphenyl-1-picrylhydraizil (DPPH).

The method is the one proposed by Brand-Williams et al.[15] with some modifications.'

Preparation of the radical DPPH: A 0.1 mM DPPH solution was prepared, weighing 3.9 mg of DPPH in a previously tared volumetric flask and dissolved in 100 mL of methanol, the solution was placed in a sonicator to ensure good dissolution and then check that the absorbance at 517 nm is between 0.9 and 1.1. The flask was covered with aluminum foil for protection from light.

Measurement of antioxidant activity: 2.9 mL of the DPPH solution was added in a cuvette and its absorbance was measured at 517 nm and then 0.1 mL of the trolox / extract dilutions were added, it was stirred vigorously and kept in the dark for 30 minutes at room temperature, and then read on a UV / VIS spectrophotometer at 517 nm.

Reaction method with the radical 2,2'-azino-bis-(3-ethylbenzthiazoline-6 ammonium sulfonate) (ABTS)

It was carried out according to the method proposed by Re R. et al.[3] using the antioxidant capacity of ABTS +· and its ability to sequester long-lived radicals. Preparation of rectifier: 0.0504 g of crystallized ammonium salt of ABTS are weighed and dissolved in 5 mL of ultra-pure water, then 6.7 mg of potassium persulfate (K2S2O8) is added and left stirring for half an hour protected from light, after this time it is transferred to a 10 mL volumetric flask and made up to the mark with ultra-pure water and allowed to react at room temperature and protected from light for 12 to 18 hours. After the time has elapsed, an aliquot of 1 mL is taken and 70 mL of phosphate buffer pH 7.1 is added and the absorbance at 734 nm is measured, which must be between 0.680 ± 0.2. To measure the antioxidant activity, 2 mL of the ABTS radical was taken in a cuvette and its initial absorbance at 734 nm was measured with the equipment thermostatted at 37 °C, then 50 µL of the trolox dilutions / SLDC aqueous extract were added (which must have been in a water bath at 37 °C), mixed for 10 seconds in a vortex, after 4 minutes of incubation the final absorbance was measured at 734 nm15. All samples were analyzed in triplicate.

Evaluation of the hepatoprotective effect

For the study, 36 adult male Holtzman strain albino rats, two months old, whose weights were between 180 and 230 g were used. They were placed in cages, in an environment at constant temperature, with alternating cycles of 12 hours of light, 12 hours of darkness, and were fed with commercial food and water ad libitum.[16] Experimental method. The experimental animals were randomly distributed into six groups of six animals each: Group I (normal control) and group II (APAP control) were administered distilled water. Groups III, IV and V were administered by intragastric route the aqueous extract of SLDC at doses of 100, 200 and 400 mg / Kg respectively, while the animals of group VI were administered silymarin (SIL) at doses of 100 mg / Kg, once a day for 7 days. On the eighth day, after the administration of the respective treatments, all the animals in groups II, III, IV, V and VI were administered paracetamol (APAP) at a dose of 2 g / Kg. The next day all rats were bled by cardiac puncture for biochemical tests.

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Biochemical tests

Blood samples were collected, centrifuged at 3000 rpm for 10 minutes to obtain serum, which was then subjected to the determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels.

Determination of aspartate aminotransferase (AST).[17]

Process

In a cuvette kept between 30-37 °C, place: 0.80 mL of Reagent A plus 100 µL of Sample. Pre-incubate for a few minutes, then add 0.20 mL of Reagent B. Mix immediately and simultaneously trigger the stopwatch. Wait 90 seconds and read the initial absorbance and then 1, 2 and 3 minutes after the first reading. Determine the average difference in absorbance / min (A / min), subtracting each reading from the previous one and averaging the values. Use this average for calculations.

Calculation of the results

GOT (U / L) =A / min x factor

Factor (30-37 °C) =1,746

Determination of alanine aminotransferase (ALT)

Statistic analysis

After the execution of the experimental design, the data were ordered and analyzed, first applying the normality test, to select the appropriate statistical hypothesis tests. As the sample did not have a normal distribution, the Kruskal-Wallis test was applied, to compare means of more than two groups, and the Mann-Whitney test, to compare means of two groups. The p value <0.05 was used to consider a statistically significant difference. For the statistical analysis, the SPSS version 2018 program was applied.[18]

Results

Tables 1–4, Figure 1, 2.

Table 1 Yield of the aqueous extract of Spilanthes leiocarpa DC

Sample weight	Extract weight	Performance %	Average
160.0g	40.1g	25.06	
160.3g	39.3g	24.51	25.08 ± 2.33
160.4g	41.2g	25.67	

Table 2 Phytochemical screening of the aqueous extract of Spilanthes leiocarpa DC

Metabolite	Results	Fractions
Flavonoids	+	A, D, E
Free amino groups	+	TO
Steroids / triterpenoids	+	B
Alkaloids	+	C
Leukoanthocyanidin	+	FROM

+ Sign indicates presence, - sign indicates absence.

Table 3 Result of antioxidant activity expressed as TEAC (1mM trolox)

Compound	Methods		
	FRAP	DPPH	ABTS
SLDC (mg)	1.76±0.35	3.21±0.37	2.81±0.19
SIL (mg)	2.25±0.18	3.79±0.12	2.38±0.08

Average values of 3 repetitions and the standard deviation.

Table 4 Results of Transaminases in blood serum in each group according to treatment

Groups	Treatment	AST (U / L)	ALT (U / L)
I	Control	66.98±5.31	46.18±4.77
II	APAP	165.95±12.19	93.80±11.05
III	APAP + SLDC 100 mg / Kg	100.48±5.75	48.16± 7.54
IV	APAP + SLDC 200 mg / Kg	79.31±13.26	62.87±6.70
V	APAP + SLDC 400 mg / Kg	69.83±9.44	52.43±9.52
SAW	APAP + SIL 100 mg / Kg	72.24±4.22	47.18±6.87

Values are expressed as mean ± standard deviation of 6 experimental animals. APAP = paracetamol, SLDC = Spilanthes leiocarpa DC., SIL = Silymarin.

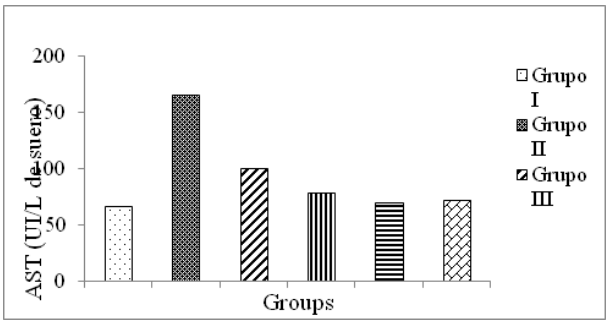

Figure 1 Results of the levels of aspartate aminotransferase in each group.

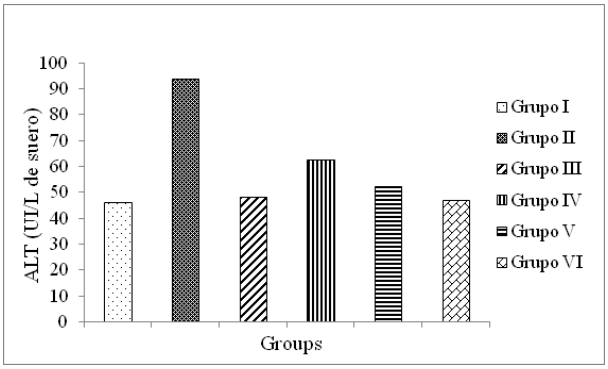

Figure 2 Results of alanine aminotransferase levels in each group.

Discussion

The metabolism of paracetamol is 90-95% at the liver level and its excretion is through the kidney, it is also a drug that, administered at high doses or chronic use, is commonly associated with hepatotoxicity and nephrotoxicity in humans and animals.[19] This process is carried out by the generation of free radicals. In this sense, the administration of substances with antioxidant properties could avoid or reduce the toxic effect of the mentioned drug. According to García[20] reports in Colombia the use of decoction flowers and juice of this plant in the treatment of liver diseases; likewise Calderón in[13] reports that in the Ica region, the entire plant is used in cooking as antiscorbutic and for liver diseases. As can be seen in Table 2, many of the fractions present groups of compounds with recognized antioxidant activities such as flavonoids, among these catechins, proanthocyanidins. In this study, the antioxidant capacity of the SLDC extract was

determined and Silimaria by different methods such as FRAP, DPPH, ABTS, said activity showed a concentration-dependent behavior in both compounds. By means of the DPPH and ABTS methods, a greater antioxidant capacity is obtained, which is explained by the mechanisms of SET and HAT, as opposed to the FRAP, whose mechanism is exclusively of SET.[11] The antioxidant activity shown by the extract of SLDC., And silymarin by means of the three methods have practically similar effects. Liver diseases are diseases related to the oxidative stress of cells and the mechanisms that attenuate these effects of free radicals are the antioxidant substances[21] in the aqueous extract of SLDC, some compounds with these characteristics that contribute to the antioxidant activity of the extract were determined.

Damage to the liver can be evaluated in various ways; one of them is the measurement of the activity of enzymes such as AST aspartate aminotrase, ALT alanine aminotrase, whose elevation in plasma or serum shows the cellular damage caused by paracetamol.[22,23] This study shows a marked elevation in serum levels of AST and ALT, in rats treated with 2 g / kg of paracetamol, indicating the generation of liver damage. However, these serum enzymes show a decrease in these values in the groups treated with the aqueous extract of SLDC, the extract with the highest activity being 400 mg / Kg, showing no statistically significant difference to the effect of 100 mg / Kg of Silymarin, these results are very similar to those obtained by Troncoso et al.[23] who evaluated the hepatoprotective effect of parsley by measuring said enzymes (AST, ALT and others) whose values decreased at the level of the control drug purinor®; however, Arnao-Salas et al.[24] tested the hepatoprotective effect of the aqueous extract of yacon, not observing an effect on the level of AST and ALT, but concluding that it had a hepatoprotective effect similar to the drug Silymarin in other enzymes, this indicates that the hepatoprotective mechanisms are diverse.

The presence of flavonoids in the aqueous extract of the Spilanthes leiocarpa DC plant. would be responsible for its antioxidant activity and hepatoprotective effect, which could act by sequestering the free radicals generated during the metabolism of paracetamol; However, it should be taken into account that the antioxidant activity in vitro is not necessarily a reflection of said effect in vivo since the compounds undergo a series of biotransformations that affect said activity.[25] These studies provide a scientific basis for understanding one of the possible mechanisms of action of Spilanthes leiocarpa DC. "Phlegm", in the use of traditional medicine and open the way to future research.

Conclusion

Phytochemical screening of the aqueous extract of Spilanthes leiocarpa DC. "Phlegm" presented the following groups of secondary metabolites: flavonoids (proanthocyanidin catechins), free amino groups, triterpenes and steroids, and alkaloids. The antioxidant activity by the FRAP, DPPH and ABTS methods of the aqueous extract of Spilanthes leiocarpa DC. and Silymarin, have a similar effect. The hepatoprotective effect of the extract of Spilanthes leiocarpa DC., At a dose of 400 mg / Kg shows a similar effect to Silymarin at 100 mg / Kg.

Acknowledgments

None.

Conflicts of interest

The autor declares there is no conflict of interest.

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