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In Number 1st presented an article Antioxidant activity jobo's pulp (Spondias mombin L.) *by REYES-MUNGUÍA, Abigail, DELGADO-GONZÁLEZ, Paola, MARTINI-MORALES and Sasi Elibeth with adscription in the Universidad Autónoma de San Luis Potosí, in the next Section an article* Analysis of proteins related to oxidative stress in Alzheimer's disease *by MINJAREZ, Benitos, RODRÍGUEZ-YÁÑEZ, Yury, MENA-MUNGUÍA, Salvador and LUNA-ÁRIAS, Juan Pedro with adscription in Universidad de Guadalajara, School of Pharmacy and Pharmaceutical Sciences & Trinity College Institute of Neuroscience, Instituto Politécnico Nacional, in the next Section an article:* Effects of NBeylax functionalized TiO_2 nanoparticle administration on the DNA of cancer cells by *ARTEAGA-LÓPEZ, Paola R., ALBARRÁN-MENA, León, LEÓN-GUTIÉRREZ, Sergio and LEÓN-GUTIÉRREZ, Gabriela with adscription in the Department of Development and Innovation GRESMEX,* in the next Section an article Evaluation of anti-inflammatory, anti-nociceptive and toxicological effects of hydro-alcoholic extract of C. pulverulentus activity in a murine model *by ALVARADO, Brenda, OLVERA-GONZÁLEZ, Vicente, HERNÁNDEZ-AGUILAR, Jaime and LEÓN-BUITIMEA, Ángel with adscription in the Universidad Autónoma de San Luis Potosí.*

Content

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Antioxidant activity jobo's pulp (*Spondias mombin L.)*

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Abstract

The aim of this study was to determine the physicochemical properties and antioxidants Jobo (*Spondias mombin L.*), and evaluated during processing and storage. Measurement of antioxidant capacity was conducted by the method described by Brand-Williams et al. (1995) the total phenolic content was determined by the Folin-Ciocalteu technique, flavonoids, carotenoids and vitamin C (spectrophotometry). In fresh pulp phase separation (supernatant and supernatant) was performed, the results show that the supernatant phase redox potential recorded 373.3 ± 0.188mV, polyphenols 298.71 ± 0.04 mg GSD / L and 77.98% inhibition of free radicals while the supernatant phase threw a redox potential of 319.5 mV ± 0.532, 57.92 ± 0.05 mg polyphenols EAG / L and 46.69% inhibition of free radicals. Flavonoids was reported in fresh pulp of 139.68 ± 0.422 mg C / g, 39.91mgAA / L vitamin C and 11.53 mg / g of total carotenes. The color intensity, ° Brix and pH were maintained without significant change. The antioxidant properties of the phases plum pulp were attributed to polyphenols.

Antioxidants, polyphenols, flavonoids, free radicals.

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Introduction

Nowadays, fruits rich in polyphenolic compounds, are attributed a high antioxidant potential, associated with several pharmacological properties related to diseases produced by reactive oxygen species, inducers of leukemia and colon cancer, among others (Sziliszka and Krol, 2013; Wang et al., 2012).

One of them is *Spondias mombin L*. commonly known as jobo, small fruit of elliptical form of 3 to 4 cm of length belonging to the family Anacardiaceae, is cultivated in tropical zones of America, Asia Africa, Brazil and Mexico, in the latter one They know two native species of that family; S. mombin L. and S. purpurea L. mombin variety. Although this species has been introduced to the crop for commercial purposes, knowledge of nutritional value is extremely scarce, considering that there is a great interest in healthy foods of a natural origin today (Sloan, 2003).

Several studies have shown that the consumption of fruits rich in antioxidants has a beneficial effect on health and contributes to the prevention of degenerative processes, particularly atherosclerosis and cancer (Temple, 2000; Hashimoto et al., 2002; Gundgaard et al., 2003). , Gosslau and Chen (2004). This is because the polyphenols, tannins, flavonoids and phenolic acids present in many fruits are able to trap free radicals that cause oxidative stress and reduce the probability of suffering from chronic diseases (Crozier et al. 2006; Contreras-Calderón et al., 2010), so that in recent years has insisted on an abundant consumption of these natural products.

High intakes of fruits and vegetables are associated with maintenance of health and prevention of diseases caused by oxidative damage such as heart disease, hemiplegia and cancer (Robbins, 2003).

For this reason, this research aims to determine the physicochemical and antioxidant properties of the jobo (*Spondias mombin L*.), and evaluate them during its processing And storage.

Materials and methods

Ssample collection

Jobo fruits were harvested in family gardens of Cd. Valles, San Luis Potosí, Mexico. A selection of the fruits was made, taking as a basis those freshly cut fruits that had a uniform yellow color and excluded those that presented bruises, detachments in the peel and those that were in state of maturation outside the normal range (dark coloration).

Obtaining pulp process

To obtain the jobo pulp the husk and seed was separated manually by means of a sieve, once the pulp was obtained it was placed in a freezer to keep it fresh and to avoid a fermentation process, later 20 ml of pulp of jobo Which was centrifuged at 3500 rpm in order to obtain the separation of the two phases of the pulping phase and supernatant phase to which they were analyzed, the percentage of free radical inhibition, polyphenol content, pH, redox potential, Flavonoids and carotenoids.

Total phenol content

The method of Folin Ciocalteu (Singleton et al., 1999) was used in which the intensity of the color produced when this reagent reacts with the phenolic compounds is measured. Absorbance readings were performed on a spectrophotometer (Aqua Mate PlusUv-Vis). 1 ml of the extract of the extract of the diluted jobo pulp phases was placed in a test tube, adding 5 ml of diluted reagent (1:10) of Folin-Ciocalteau, letting stand for 8 min and then adding 4 ml.

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Of the 7.5% sodium carbonate solution until a homogeneous mixture is obtained. The tubes were covered with aluminum to protect them from strong light and incubated for 2 h at room temperature. They were then read at 740 nm. Results were expressed as milligrams of Gallic Acid Equivalents per liter (mg EAG / L).

Redox potential

Measurements were made with a platinum electrode and an Ag / AgCl reference electrode connected to a potentiometer (Orion, 720 A). Calibration was performed against a standard redox solution (E = 20 mV at 25 ° C). The electrodes were introduced into a 50 ml beaker containing 30 ml of extract of the phase extract from the jobo pulp. The redox potential values in mV were recorded for at least 5 min, so that the redox potential reached stability. A stable potential is arbitrarily defined as a change of less than 1 mV over a period of 5 minutes (Reyes et al., 2009).

Percentage free radical inhibition

To determine the percent inhibition of free radicals, 3 ml of DPPH were placed in a cell and 100 µl of diluted jobo pulp phases were added. The mixture was allowed to react in the dark and the initial and final change in absorbance (Thermo Scientific Aqua Mate Plus mono / UV beam spectrophotometer) was monitored at a wavelength of 515 nm of the samples for a period of 30 minutes each 5 minutes at 25 ° C. The percent inhibition DPPH • was calculated according to equation 1.

% Inhibition of free radicals = initial Absorbance - final Absorbance x 100

Flavonoids

The reagents prepared were 5% sodium nitrite (NaNO2) with distilled water. 10% aluminum chloride (AlCl3) with distilled water and 1M sodium hydroxide (NaOH). The standard curve was made with 150 to 1000 µm catechin points with methanol (80%). The method used was 250 µl of sample from the jobo pulp phases plus 1.25 ml of distilled water, 75 µl of 5% NaNO2 was added and allowed to stand for 5 minutes, then 150 µl of AlCl 3 was added % And allowed to stand for 6 minutes and finally 500 µl of 1M NaOH and 275 µl of distilled water were added. Each of the samples was immediately analyzed at 510 nm of absorbance in a spectrophotometer. The data from this analysis were expressed in mininquivalent catechin per gram of sample (Wolfe et al., 2003).

pH

The pH was determined with a potentiometer (Thermo Scientific Orion Dual Star®) calibrated with buffer solutions of pH 4, 7 and 10 at 25 ° C. Measurements of the sample were then performed by placing 30 ml of the extract of shell and phases of the jobo pulp in a 50 ml beaker, to perform the readings according to the methodology of (Reyes et al., 2009).

Total solids content

Two drops of fresh jobo pulp sample were placed on the Reichert Brix 35 HP refractometer by performing two replicates according to the International Association of Analytical Chemists (AOAC) method to measure ° Brix (Reyes et al., 2010).

Quantification of total carotenoids

The pigment extraction procedure was carried out under dark conditions. 0.5 g of jobo pulp were weighed and 20 ml of acetone-ethanol-hexane extraction solution (1-1-2 v / v / v) were added.

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The flasks were shaken for 15 min and 3 ml of distilled water were added. Stirring was continued for 15 min and allowed to stand to allow phase separation. The organic phase was separated (above) and stored protected from light. 20 ml of an extraction solution was added again and the process was repeated until phase separation. The organic phases were combined and read at 503 nm (Olivera et al., 2012).

The concentration was calculated using the molar absorption coefficient for β carotene.

Quantification of vitamin C

The reagents prepared were 0.4% oxalic acid (C 2 H 2 O 4), 2.9 mg of the dichloroindophenol salt (DIP) were weighed into analytical balance to obtain a 500 μm solution and was made up to 20 ml. From the above salt a DIP solution at 50 μm was prepared, taking 2 ml of the solution at 500 μm and dosed at 20 ml. The method used was, the blank was read with distilled water, 300 μl of 0.4% oxalic acid + 2700 μl DIP 50 μm were measured in a cell and left to stand for fifteen seconds (record this data to be used for the L1 calculations), Was adjusted to zero with 300 μl of pulp sample + 2700 μl distilled H 2 O, and 300 μl of pulp sample + 2700 μl DIP (log L 2), L 1 - L 2 (with or ascorbic), (Hung and Yen , 2002).

Statistic analysis

The data obtained were processed using the STATISTICA 8 program to obtain the mean and standard deviation. All determinations performed in this work were carried out in triplicate.

Results and discussion

Antioxidant compounds and vitamins are naturally present in fruits, vegetables, herbs and spices (Lu et al., 2008).

The content of these varies considerably depending on the part of the fruit analyzed, the maturation stage, cultivation and processing practices and even the hours of light as they influence the synthesis of the bioactive compounds (Gayosso et al., 2011).

Flavonoids

In the extract of jobo pulp was reported a Flavonoid content of 139.68 ± 0.422 mg C / g. (Table 1). When analyzing the relation of the values of flavonoids obtained with the content of total polyphenols can be said that they are within the expected since the flavonoids are only a subgroup of the polyphenolic compounds. The content of flavonoids in S. mombin reported in this research is higher than that reported by sour guava (Psidium araca) of 133.73 ± 1.12 mg C / g (Zapata et al., 2013), which indicates that the extract of Jobo pulp is an important source of antioxidant activity (Watson and Preedy, 2010).

Vitamin C

Vitamin C is considered to be the main natural contribution of an antioxidant compound in the diet (Almeida et al., 2011). The fruits of the genus Spondias are generally not recognized as important sources of vitamin C compared to other tropical fruits such as lychee and guava. In this work, the pulp extract of *Spondias mombin L*. recorded 39.91 mgAA / L (Table 1), slightly higher than that reported for (Spondias purpurea L.) of 34 mgAA / L. (Koziol and Macía, 1998); Some of the factors that could influence the amount of ascorbic acid obtained could be the type of crop, the time in which it was made, the temperature, and the degree of maturity of the fruit harvest, because they can accelerate the oxidation of the ascorbic acid. Ascorbic acid and thereby decrease its concentration (Lee and Kader, 2000).

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Carotenoids

The values obtained in the quantification of carotenoids are shown in Table 1, a concentration of 11.53 mg / g of total carotene was obtained in fresh jobo pulp. These results are higher than reported by Robles et al. (2007) who report a content of 6.8 mg / g of total carotene for mango pulp under storage conditions.

Sample	Flavonoids (mg catequina/g)	Vitamin C (mg Ac. Ascórbico/L)	Carotenoids mg/g de muestra
Fresh jobo pulp extract	139.68± 0.422	39.91 ± 0.021	11.53 ± 0.035

Table 1 Antioxidant capacity of the fresh extract phases of the jobo pulp.

Evaluation of the antioxidant properties of the jobo (*Spondias mombin L.*), in the swelling and supernatant phases in fresh pulp.

In order to process the sample effectively the separation of the extract of the jobo pulp with which the phases were obtained; And this supernatant is often ignored because it has a higher density, the swelling phase was also analyzed because the reducing power may be superior to the supernatant phase due to the presence of antioxidants associated with cell walls and macromolecules.

YAccording to the results obtained, it was found that the swelling phase presents higher antioxidant activity compared to the supernatant phase of the jobo extract. This can be observed in the high polyphenolic content (298.71 ± 0.04 mg EAG / L), related to the percentage of inhibition of the DPPH radical (77.98 ± 0.07%), and the redox potential of 366.4 ± 0.532 mV. (Table 2).

Percentage free radical inhibition

En la figura 1 se presenta el tiempo de almacenamiento con respecto a la actividad antiradicalaria en ambas fases del extracto de jobo.

During the first days the laboring phase of the jobo extract obtained a 40.12% inhibition of Free radicals compared to what is recorded in the supernatant phase. There was a tendency to decrease as storage time advanced. This is because both phases underwent oxidation, which generated partial loss of antioxidant capacity. Oxidation could occur by the action of environmental factors such as heat moisture, light air, as reported by Mirhosseini et al., 2008. It has been shown in a large number of studies that antioxidant compounds are very susceptible to oxidation reactions when exposed to oxygen, light, moisture and high temperatures (Durst and Wrolstad, 2001, Rein, 2005, Gil et al., 2006).

Muestra	Polifenoles (mg EAG/L)	%Inhibición radicales libres	Potencial redox (mV)
Sobrenadante del extracto de jobo	57.92± 0.05	46.69 ± 0.03	355.5 ± 0.532
Fase nadante del extracto de jobo	298.71±0.04	77.98 ± 0.07	366.4 ± 0.532

Tabla 2 Propiedades antioxidantes en la fase nadante y fase sobrenadante del extracto fresco de jobo (*Spondias mombin L).*

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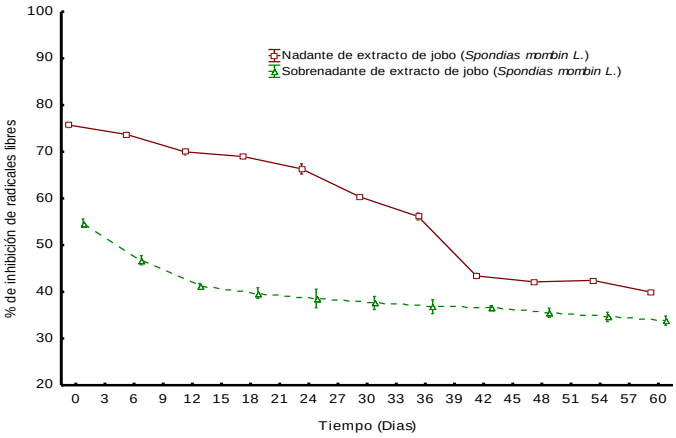

Figure 1 Comparison of the degradation kinetics in the swimming phase and supernatant phase of the jobo pulp (Spondias mombin L.). Regarding the reaction time with the radical DPPH.

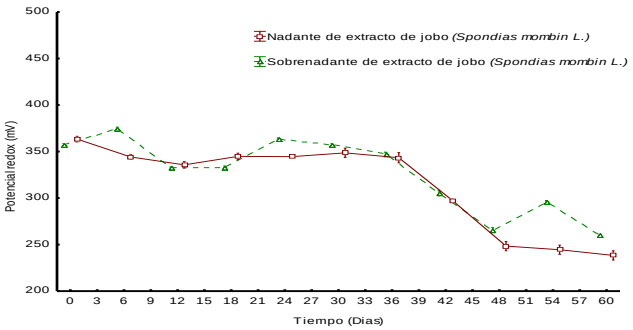

Figura 2 Comparación del contenido polifenólico de la fase nadante contra la fase sobrenadante del extracto de pulpa de jobo (*Spondias mombin L.*).

Polifenoles

Figure 2 shows the storage time on the total polyphenolic compounds quantified in the swelling and supernatant phase of the jobo extract. The initial value in the swimming phase of 298.71 ± 0.04 mg EAG / L. Decreased by 34.24% at 45 days of storage, with a statistically significant difference ($p < 0.001$) in the values of phenolic compounds. On the other hand, the supernatant phase of the jobo extract recorded an initial value of 57.92 ± 0.05 mg EAG / L. Which began to decline after 21 days of storage.

In general, during the fruit storage and processing of their products, oxidation reactions of readily oxidizable phenolic substrates occur. These reactions are catalyzed by enzymes such as phenolics, which undergo polymerization reactions resulting in dark pigments and the loss of the antioxidant capacity of the product (Robards et al., 1999). The total phenolic compounds present in the swine and supernatant phases of the jobo extract were maintained for 42 days within the range 298.71 ± 0.04 - 229.98 ± 0.03 mg EAG / L, higher compared to that recorded by other fruits such as guava> Strawberry> pineapple of 83.1> 132.1> 21.7 mg EAG reported by Vasco et al. (2008).

Redox potencial

Both phases, nadante and supernatant of the jobo pulp extract presented variations for potential redox during storage (Figure 3). The bathing phase of the jobo extract presented a higher value of 366.4 ± 0.532 mV, during 39 days of storage; However at day 42 a reduction of its reducing power equivalent 68.5% was presented in relation to the content of polyphenols and the% inhibition. These values are higher than those reported by Martínez (2012), where the extract of yellow plum pulp obtained a reducing energy of 362.23mV. While the supernatant phase reported a redox potential of 355.5 ± 0.532 mV lower than the value obtained in the swimming phase. The redox potential indicates the amount of oxidation and reduction in a solution. The higher redox potential the greater the oxidation capacity, therefore the greater the ability to yield electrons and inactivate oxidizing agents. Otherwise, the lower the redox potential, the lower its reduction capacity. In this study it can be observed in the obtained values that both samples contain high redox potential which evidences a high antiradical activity.

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pH

PH is a factor that regulates chemical, biochemical and microbiological reactions. It affects functional properties such as: color, taste and texture of food. The swelling phase of the jobo pulp extract reported a pH of 2.20 plus acid in comparison with registered By the supernatant phase of 2.81 (Figure 4), these data are similar to those reported for Spondias purpurea L. of pH 2.30,

Koziol and Macía. (1998). The acidic media in both extracts may be due to a higher content of H + ions, which could be indicative of the presence of a large number of phenolic compounds (Wade, 2004), and this can be proved by the redox potential measurements performed.

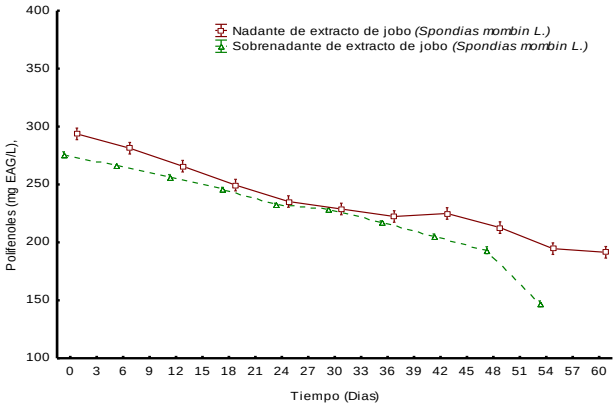

Figure 3 Comparison of the redox potential of the swimming phase against the supernatant phase of the jobo pulp extract (*Spondias mombin L.*).

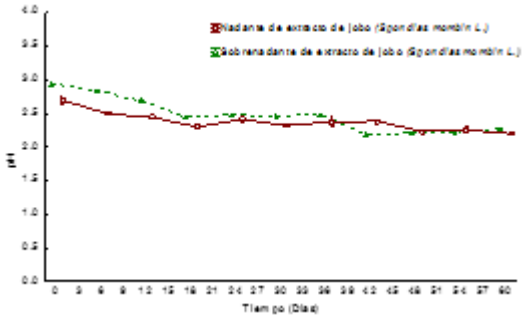

Figure 4 Comparison of pH levels in the swimming phase and supernatant phase of the jobo pulp (*Spondias mombin L.)*

It is important to note that the data obtained in this study when compared to other fruits such as the apple Malus spp and the tuna Opuntia spp. PH 3.9 and pH 6.4 Pepper (1990), notes that the jobo contains a greater amount of polyphenolic compounds which, when possessing a reducing character tend to present a pH acid which is clearly evident in this study. The lowest pH value of the swimming phase and the supernatant reached it at 48 and 42 days respectively, the swim phase ended with a pH of 2.38 and the supernatant phase with a pH of 2.43.

Total Solids

In the natural ripening process, most fruits experience a decrease in acidity and an increase in total solids levels (Osterloh et al., 1996). In this investigation it was observed that in both phases the total solids did not change in relation to the time of storage (Figure 5). The supernatant phase of the fresh jobo extract reported a lower soluble solids content (9-10 ° Brix), compared to the fresh jobo (12-10 ° Brix) seedling phase; This result is similar to that reported by Dias et al. (2003), where they refer values of soluble solids in fresh jobo pulp close to 12 ° Brix.

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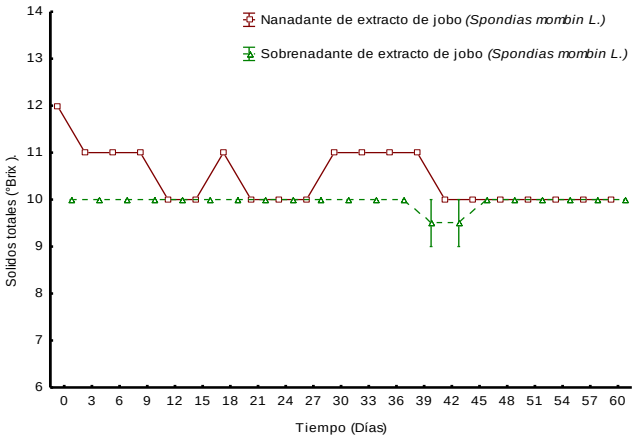

Figure 5 Comparison of the levels of ° Brix in the swimming phase and supernatant of jobo (*Spondias mombin L.).*

The trend observed was that as the total solids increased, a higher antioxidant capacity was present, a fact that could be related to the increase in the content of free polyphenols acting as free radical scavengers. The correlation analysis of the antioxidant activity in the supernatant and supernatant phase of the jobo pulp extract indicated that there is a direct association between polyphenols and antioxidant capacity, since its correlation coefficient for the swelling phase was R = 0.9304 (Figure 6 A) and for the supernatant phase was R = 0.9377 (Figure 6B), indicating that the concentration of polyphenols in both phases of the fresh jobo extract constitute an important source of antioxidant activity. Fuhrman et al. (2001) argue that the antioxidant capacity of white wines is directly proportional to their polyphenol content, likewise Yen et al. (1993) report that the antioxidant activity of the extracts is mainly related to the concentration of polyphenols since they find a correlation coefficient between the antiradical activity and the concentration of polyphenols of R = 0.967.

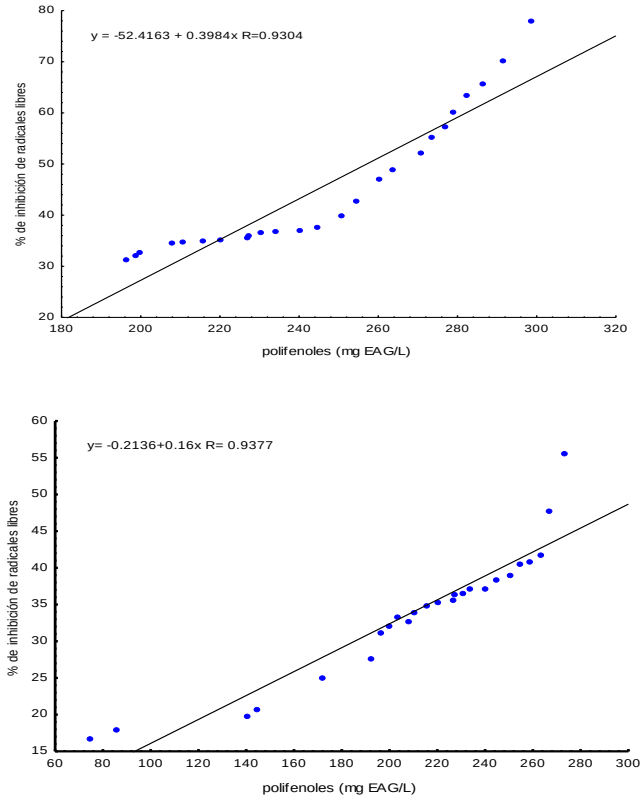

Figure 6 Correlation curves of fresh extract of jobo pulp *(Spondias mombin L*). A) Correlation between% inhibition of free radicals and polyphenols of the swimming phase. B) Correlation between% inhibition of free radicals and polyphenols of the supernatant phase.

Conclusions

Based on the results obtained, it is possible to conclude that both phases of the jobo pulp extract, besides containing a large number of polyphenolic compounds, also present other components such as flavonoids that give it the antioxidant capacity.

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The above is reflected in the results of the phases of the extract where it was obtained that measurements of the swimming phase of the extract of jobo, presented greater polyphenolic content of 298.71 ± 0.04 mg EAG / L, redox potential of 366.4 ± 0.532 mV and activity Antirradical ratio of 77.98 ± 0.07 compared to the supernatant phase that reported polyphenolic content of 57.92 ± 0.05 mg EAG / L, redox potential of 355.5 ± 0.532 mV and 46.69 ± 0.03% of free radical inhibition.

It is also concluded that as the soluble solids increase a higher antioxidant capacity, which could be related to the increase in the content of free polyphenols acting as free radical scavengers.

Because there was a direct correlation between the values of polyphenols, flavonoids, carotenoids, vitamin C, total redox potential, with antiradical activity in both phases of the extract, the results obtained allow to recommend to jobo as a potential source of natural antioxidant for Human diet confirming its beneficial effects on the health of the population.

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Analysis of proteins related to oxidative stress in Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is the commonest cause of dementia in the elderly corresponding to approximately 60 to 80% of all cases. At the neuropathological level, AD brains are characterized by the presence of neurofibrillary tangles (NFTs) and neuritic plaques. NFTs are constituted of paired helical filaments (PHFs), which are structurally composed by assembled hyperphosphorylated and truncated Tau polypeptides. AD is considered the result of complex events involving both genetic and environmental factors. To date, the molecular mechanisms underlying the etiology of this devastating disease, have not been fully unveiled. But some pathological features of AD include oxidative stress and mitochondrial dysfunction. Thereby, the aim of this study was to identify those proteins that are involved in these kind of processes. Particularly, those non-previously described as connected with the disease. We can correlate the oxidative stress caused by ROS directly affecting mitochondria, allowing us to test the relationship of oxidative stress and neurodegeneration along the pathological process of AD.

Alzheimer, Mass spectrometry, ROS and Oxidative stress.

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Introduction

Alzheimer's disease (AD) is a progressive, degenerative and irreversible pathology, being the most common cause of dementia in the elderly. In 2010, about 35.6 million people with AD were estimated around the world; However, this figure has been forecast to double every 20 years, with 65.7 and 115.4 million people in 2010 and 2050, respectively (Wimo et al., 2013).

AD is a complex and multifactorial disease, where it is possible that some of the structural alterations of this type of dementias develop in the brain tissue in the years prior to its clinical manifestation (Korolainen et al., 2010), which include the decrease Of cognitive abilities, neuronal and synaptic loss in specific areas of the brain, and an evident loss of memory (Lee et al., 2001, Gotz and Ittner, 2008, Querfurth and LaFerla, 2010).

For this reason, AD is considered to be an age-dependent disease and given the increasing aging of populations, this pathology constitutes a serious public health problem, as several studies indicate (Mayeux and Stern, 2012). In addition, another important point to consider is the use of a large amount of resources, both economic and human, in the care and treatment of these patients. For example, during 2010 an investment of more than 600 billion dollars was made only for the care of patients with AD around the world (Prince et al., 2011).

Patients present two major histopathological features, the presence of extracellular deposits of the amyloid beta peptide (Aβ) forming the so-called neuritic plaques (PNs), and the intracellular accumulation of filaments known as "neurofibrillary tangles" (MNFs) by the hyperphosphorylated Tau protein (Selkoe, 1991; Trojanowski et al., 1995). Such accumulations result in neuronal dysfunction and cell death.

Given the large accumulations of MNFs and PNs, AD is considered to be a disease related to the "deregulation of misfolded proteins" or "misfolding" (Nussbaum et al., 2012). In addition, this disease is often accompanied by microvascular damage and important inflammatory processes in different regions of the brain (Bertram et al., 2011).

Alzheimer's disease can occur either sporadically or in a family or hereditary way. Most of the cases belong to the sporadic form and represent more than 90% of the patients. 10% of the remaining cases are of the family type and may manifest early or in adulthood. It is possible to emphasize that the familiar Alzheimer behaves in a very similar way to the sporadic form; however, familial Alzheimer's presents a more aggressive symptomatology (Arango-Lasprilla et al., 2007; Benitez et al., 2013).

Although the precise etiology for AD is unknown, some risk factors such as aging, the presence of some susceptibility genes, the environment and metabolic problems have been described. However, the most recurrent processes are those related to oxidative stress, impaired energy metabolism, mitochondrial damage and disturbance of metal homeostasis (Mattson, 2004; Eckert et al., 2010).

Oxidative stress in AS.

Oxidative stress as already mentioned is one of the main factors involved in the pathological progression of AD (Smith et al., 1997; Butterfield et al., 2001). Abnormal amounts of proteins, lipids, and DNA modified by oxidative processes have been shown in brain tissue from patients with this disease, suggesting the role of this phenomenon in Alzheimer's evolution (Butterfield et al., 2001).

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To date, some sources have been proposed that generate oxidative stress in AD, among them A acum accumulation and alteration in cellular redox activity by some metals such as Fe2 + and Cu2 + (Smith et al. 1997; Butterfield et al., 2001), the latter being a very conserved and important process in the typical processing of the disease.

In addition, there is evidence that iron and its regulatory proteins are involved in the mechanism of many neurodegenerative diseases, since some mutations in genes encoding these proteins are related to iron metabolism (Zambenedetti et al., 2003; Lehmann et al. , Which usually accumulate in the same regions that are affected in AD (Choi et al., 2004).

This makes more evident the role of oxidative stress and mitochondrial damage, as a consequence of the decrease in energy balance, thus maintaining the pathological processing of AD. The presence of free radicals and reactive oxygen species (ROS) favor oxidative imbalance by increasing neuronal degeneration and inflammatory processes (Anastasio, 2011).

ROS and AD

Although ROS are produced naturally during cellular respiration, they are usually kept at relatively low levels thanks to the action of different antioxidant enzymes. Unfortunately, sometimes this control mechanism can be damaged or overcome, preventing the cell from dealing with these molecules, thus allowing its accumulation, generating damage and imbalance in cellular homeostasis (Amandine et al., 2016).

Among some of the proteins related to ROS is the participation of the enzymatic family of glutathione S-transferases (GSTs). In this sense, recent studies have suggested their participation in antioxidant enzymes against free radicals, as well as detoxifying against other toxic agents.

Because these dimeric enzymes catalyze the deprotonation of glutathione (GSH) and form a thioether bond with electrophilic substrates, the solubility and excretion of these agents is increased, acting as a defense mechanism against ROS and oxidative stress (Hayes and Pulford, nineteen ninety five) .

GSTs are grouped into seven families (alpha, kappa, mu, pi, sigma, theta and zeta), each presenting a difference in its amino acid sequence and therefore in its biological functions (Mazzetti et al., 2015). In particular, GSTP has been linked to several pathologies such as cancer and neurodegeneration (Vasieva, 2011), as has been shown to play an important role in the detoxification of a wide variety of diseases, including Alzheimer's (Bolt, 2006). In addition, GST is essential in various cell signaling pathways, emphasizing apoptosis, cell proliferation and regulation in response to stress (Cho et al., 2001).

Mass spectrometry in the AD.

Although there is a great advance in the knowledge and understanding of the mechanisms involved in the pathological processing of AD, the precise diagnosis in many cases remains uncertain until death (Koren et al., 2009). For this reason, new strategies are needed to characterize the etiology of this disease and thus identify and propose specific biomarkers that can be used in early diagnosis or indicate risk factors, presence and progression (Zellner et al., 2009). Recently, new tools have been developed for the detection and identification of proteins from enriched samples. The most widely used method is mass spectrometry (MS) coupled to liquid chromatography (LC), through which it has been possible to identify some candidate markers for the diagnosis of AD (Wang et al., 2005; Minjarez et al. , 2013).

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Previously, we report the use of this technology and the identification of more than 100 proteins, some of them previously documented by other authors, which include, just to name a few, GAPDH, which has already been linked with AD as a Possible biomarker, UCHL-1, related to oxidative stress, and transferrin, which has been characterized as an iron regulatory protein (Minjarez et al., 2013). However, the methods used at that time were only qualitative, so it is not possible to know the different levels of expression between the different cases. For this reason, a quantitative analysis was required.

For this, the use of quantitative proteomics tools such as the specific labeling tests offered by the iTRAQ system (Isobaric Peptide Tags for Relative and Absolute Quantification) can provide a further advance in the identification and quantification of complex samples in a single experiment and Thus knowing the expression levels of these proteins in each of the analyzed samples (Melanson et al., 2006). This method is based on the labeling of peptides by means of labels that are covalently and differentially bound, and in a single experiment can mark 4 or up to 8 different samples from a previous enzymatic digestion (Melanson et al., 2006).

It should be noted that although several studies have been carried out with this technology for the identification of biomarkers that help the diagnosis of several pathologies such as Parkinson's disease, dementia by Lewy bodies, in addition to AD, these have been performed using different types Of samples, including those obtained from cerebrospinal fluid (CSF) (Abdi et al., 2006) or from different animal models such as transgenic mice (Zhang et al., 2008; Rhein et al., 2009), as well as cellular models (Zhang et al., 2008).

These studies allow us to know to some extent the expression profiles of the different proteins expressed in the models evaluated; However, it is considered necessary to perform such studies directly on human brain tissue since it represents a profile closer to that found in patients with this disease (Rudrabhatla et al., 2010). Thus, we previously reported the expression profiles of proteins in brain tissue of patients with AD compared to a case control using quantitative proteomics using iTRAQ labeling and mass spectrometry (Minjarez et al., 2016).

In this study we identified more than 700 proteins from which, we selected those that showed in the 3 cases Alzheimer type an expression change of at least 20% compared to the control case. Of these, 61 and 69 proteins were classified as overexpressed and underexpressed, respectively. Subsequently, three different proteins (PGM-1, RHO, Sideroflexin) were selected and evaluated by means of interference RNA in in vivo models of transgenic flies expressing A□ or Tau, in order to know the impact that generated both in Anatomical aspects as functional in the eye of these flies when the expression of some of these genes was inhibited (Minjarez et al., 2016).

Functional analysis of these genes produced clear evidence of two characteristics related to AD: 1) Mitochondrial function is significantly affected, and 2) there is an alteration in the expression levels of the proteins involved in the redox balance and the regulation of ROS. In this way, a large variety of proteins were identified both qualitatively and those that changed their level of expression in brains with Alzheimer's disease. These results are closely related and support the general view of this disease, in addition to proposing the use of Drosophila melanogaster as an in vivo model to determine the effects of new proteins on the role of key actors in AD, such as.

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Methodology

Bioinformatic analysis

For the classification of polypeptides, the PANTHER version 10.0 (Protein Annotation Through Evolutionary Relationship) classification system was used on the website http://www.pantherdb.org/, where proteins were grouped according to their function Biological and molecular (Mi et al., 2013). For the identification of the functional interaction networks, the STRING platform 10.0 (Search Tool for the Retrieval of Interacting Genes / Proteins, http://string-db.org/) was used, as well as active prediction methods Of coexpression, gene fusions, experimental, cooccurrence, databases and mentions in different publications, with a filter of high confidence (0,7). Finally, the analysis was performed using the KEGG (Kyoto Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg/) platform, which facilitates the identification of the metabolic pathways of both over- and under-expressed proteins and allows to visualize signaling cascades related to mimas.

Results

Identification of proteins related to oxidative stress, mitochondrial damage or regulatory of metals.

Previously, we mentioned some studies in the field of research for AD using tools such as proteomics and bioinformatics. These reports show the improvement of some techniques related to the complexity of the sample or to two-dimensional electrophoresis, followed by mass spectrometry, labeling with fluorescent compounds, among others. Unfortunately, many of these studies have used samples of non-human type, which may generate biases in the information obtained. However, this limitation could be avoided by using brain tissue from patients with this disease.

For this reason, we previously performed 2 different studies using this type of samples and their subsequent analysis by mass spectrometry and different bioinformatic platforms (Minjarez et al., 2013, 2016).

For the present work, the results obtained in the quantitative study were taken and by means of different platforms bioinformatics (see Methodology) selected those proteins that had some relationship with processes of oxidative stress, mitochondrial damage and regulation of metals. As a result, detection of 39 different proteins, which, were recognized by at least one peptide with a value of 95% confidence or greater. Of these, 19 proteins showed overexpression values and 20 of subexpression (see Table 1).

Once the different proteins to be evaluated were known, they were grouped and classified according to their biological and molecular function using the PANTHER classification system. It is important to note that several proteins may be grouped into more than one group because they may have more than one function. In this way, the largest number of analyzed members (23 members) had functions related to the catalytic activity, representing more than 59% of the total, followed by the binding proteins and the carriers with 6 (15%) and 5 (13%) Members respectively. It should be noted that 4 proteins with antioxidant activity were also identified, representing 10% of the total proteins analyzed (data not shown).

Subsequently, within the group with catalytic activity we identified 2 large subgroups, among which proteins with oxidoreductase activity and hydrolase, with 12 and 7 members respectively, and taking both groups represent almost 80% of the total proteins studied.

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Once the proteins are grouped according to their function, we determine a possible interrelation between each of them through an interactome. For this purpose the polypeptides were evaluated with the software STRING. We emphasize that even if we did not identify any beta amyloid peptide by mass spectrometry, we decided to include it.

This analysis yielded four main biological processes; each with a significant statistical value equal to or greater than 0.05 and with a cluster coefficient of at least 0.700 (Figure 1). The results obtained indicate that the most important biological process is the reduction oxide with a P value of 4.77x10-10 containing 18 members, followed by the oxidation energy derivation group of organic compounds with 12 identified polypeptides and a value of P of 5.27x10-9. Finally the third and fourth groups to be highlighted are those of cellular respiration and response to oxidative stress, both with 9 members and a P value of 1.39x10-7 and 3.05x10-5, respectively.

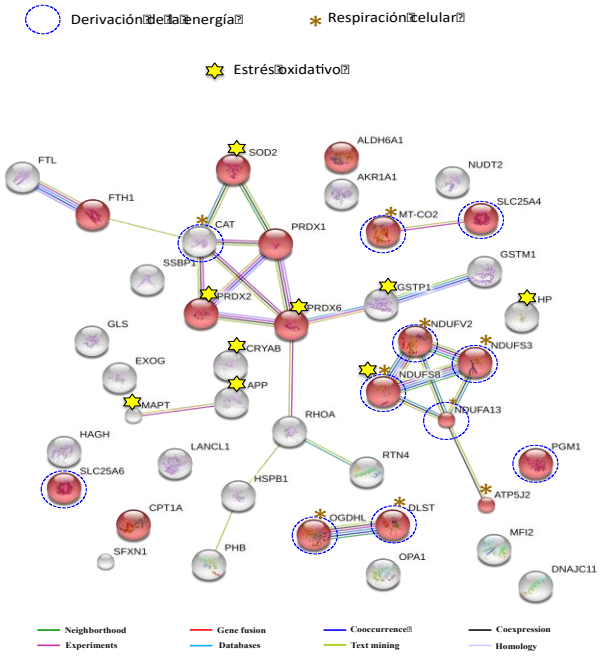

Figure 1 Interactoma of polypeptides identified by quantitative mass spectrometry studies related to some pathological event in Alzheimer's disease. The UniProtKB access numbers of each of the proteins of interest were used and analyzed using the STRING program to identify their functional network. The different lines of colors represent the different evidences for each identified interaction. Polypeptides involved in oxidation-reduction processes, derivation of energy by oxidation of organic compounds, cellular respiration and response to oxidative stress are indicated by red circle color, blue dotted circle, asterisk and yellow star respectively. Proteins had a statistical significance (p-value ≤0.05) in the different biological processes that were related.

Then, each of the proteins studied were assigned to some metabolic pathway by means of the KEGG platform. The results obtained identified 7 different proteins previously reported and related to Alzheimer's disease (Figure 2). It is highlighted that 5 of the 7 identified proteins (P00403, O75489, O00217, P19404 and Q9P0J0 from the UniProtKB database) are directly related to metabolism and share the mitochondria as their common subcellular localization, which reflects the direct relationship of this organelle with the pathological processing of AD.

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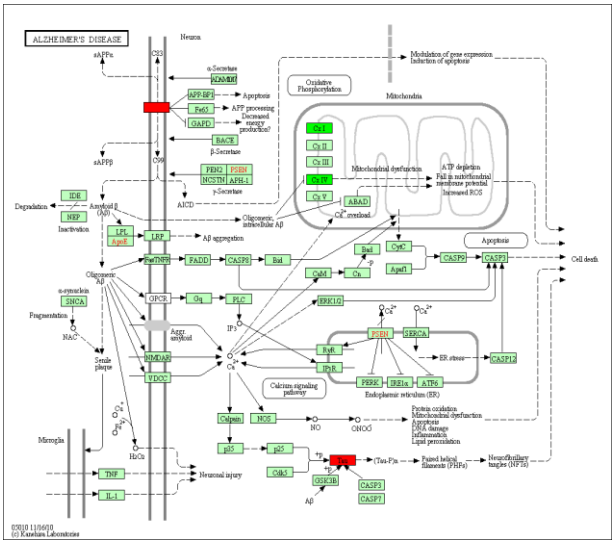

Figure 2 Identification of the metabolic pathway related to AD. The metabolic pathway for proteins previously selected and documented for Alzheimer's disease was identified from the KEGG database. The location of the subexpressed polypeptides in each of the routes are marked in bright green and the ones overexpressed in red. There are mainly five different under-expressed and related respiratory chain polypeptides.

Conclusions

In this work we have described the use of techniques of quantitative analysis and the use of bioinformatics platforms with the objective of studying and describing those proteins related to the pathological processing of one of the main neurodegenerative diseases that affect the world population as it is EA.

For this study, some previous reports describing the expression profiles of the proteins present in brains of patients for AD compared to a control (non-Alzheimer's) case were taken as reference. These studies show a marked tendency towards the deregulation of proteins related to typical events in the course of this disease, among which mitochondrial damage and oxidative stress.

There is widespread agreement that the imbalance between oxidizing agents and antioxidant factors may favor the emergence and development of a large number of diseases, including AD.

In this sense, one of the most recurrent events is the accumulation of so-called free radicals and ROS, which normally occur within cells due to their biological functions; However, their accumulation produces cytotoxicity (Butterfield et al., 2001, Choi et al., 2004).

For this reason, the deregulation of proteins related to the control and degradation of free radicals and ROS along with the regulatory proteins of metals have also been related to early events of the disease and whose presence can at the same time function as natural antioxidants (Murakami et al., 2011). In addition, homeostatic disruption of neurons and mitochondrial dysfunction result in an imbalance both energetic and metabolic, favoring the activation of different pathways, including apoptotic and inflammatory pathways.

As a result, we identified 39 different proteins whose main function was catalytic activity, emphasizing the function of oxidoreduction, as well as that of hydrolases. To know the interaction and relationship that these proteins have in relation to the amyloid beta and the Tau protein, an interacoma was obtained, which shows the participation of three different biological events, where oxidoreduction, the function of Generators and metabolic precursors and, finally, the oxidation of organic compounds.

Once we knew the main biological and molecular functions of the different proteins identified for this study, we decided to study the metabolic pathway that is affected. For this we rely on the KEEG platform, which indicates the presence of seven proteins, of which five have already been related to Alzheimer's disease, including Cytochrome c oxidase (P00403), NADH dehydrogenase alpha 1 (Q9P0J0), NADH dehydrogenase 2 (P19404), NADH dehydrogenase 3 (O75489) and NADH dehydrogenase 8 (O00217).

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All of them with direct function in the mitochondrial respiratory chain. It should be noted that these five proteins were found to have subexpression values in the 3 Alzheimer's brains compared to the control case, suggesting possible mitochondrial damage and deregulation in the energy balance.

These results have allowed us to identify those molecules with some role in redox activity and expression levels that are directly related to oxidative stress and mitochondrial dysfunction. In addition, this information is of relevance for the development and continuity of the present project, fulfilling with the purpose of knowing more the role of these proteins and its relation with the pathological evolution of the AD.

Thus, the use and application of new technologies, such as mass spectrometry and different bioinformatics platforms for the study of diseases, open a range of possibilities in the identification of proteins involved in these pathologies, as well as the biological processes involved And their interaction. In addition to doing it with a greater specificity and speed than the conventional methods hitherto used.

In this sense, the data obtained in this work provide relevant information in the search and identification of possible biomarkers in patients with AD and thereby generate a greater knowledge about the type of participating biomolecules as future therapeutic targets that help prevent the appearance and / Or the development of the earliest pathological events of this disease.

However, some of the aspects described in the present study need to be studied in more depth; However, it generates contributions to the knowledge about the pathogenesis of AD and opens the door for the use of this methodology, not only in other neurodegenerative diseases, but also in the clinical medical field in general.

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Name of the protein	UniProt KB	Gen	Protein class
Alpha-crystallin B chain	P02511	CRYAB	Chaperona
Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial	Q02252	ALDH6A1	Deshidrogenasa
Haptoglobin	P00738	HP	Serina proteasa
Ferritin light chain	P02792	FTL	Reguladora de metales
Peroxiredoxin-6	P30041	PRDX6	Peroxidasa
Ferritin heavy chain	P02794	FTH1	Reguladora de metales
Glutathione S-transferase P	P09211	GSTP1	Transferasa
Glutathione S-transferase Mu 1	P09488	GSTM1	Transferasa
LanC-like protein 1	O43813	LANCL1	Receptor
Nuclease EXOG, mitochondrial	Q9Y2C4	EXOG	Endonucleasa/ Reguladora de metales
Phosphoglucomutase-1	P36871	PGM1	Glucosiltransferasa
Reticulon-4	Q9NQC3	RTN4	Trafico de membranas
Melanotransferrin	P08582	MFI2	Serina proteasa
Peroxiredoxin-1	Q06830	PRDX1	Peroxidasa
Superoxide dismutase [Mn], mitochondrial	P04179	SOD2	Oxidoreductasa
Peroxiredoxin-2	P32119	PRDX2	Peroxidasa
Heat shock protein beta-1	P04792	HSPB1	Chaperona
Catalase	P04040	CAT	Peroxidasa
Alcohol dehydrogenase [NADP(+)]	P14550	AKR1A1	Reductasa

Table 1 Quantitative analysis of overexpressed polypeptides, identified in protein extracts obtained from brains with Alzheimer's disease, by labeling with iTRAQ and tandem mass spectrometry.

Name of the protein	UniProtKB	Gen	Protein class
1. NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial	O75489	NDUFS3	Oxidoreductasa
2. 2-oxoglutarate dehydrogenase-like, mitochondrial	Q9ULD0	OGDHL	Deshidrogenasa/ Reguladora de metales
3. ADP/ATP translocase 3	P12236	SLC25A6	Deshidrogenasa/ Reguladora de metales
4. DnaJ homolog subfamily C member 11	Q9NVH1	DNAJC11	Chaperona
5. NADH dehydrogenase [ubiquinone] iron-sulfur protein 8, mitochondrial	O00217	NDUFS8	Deshidrogenasa/ Reductasa
6. ATP synthase subunit f, mitochondrial	P56134	ATP5J2	ATP Sintasa
7. Glutaminase kidney isoform, mitochondrial	O94925	GLS	Hidrolasa
8. Cytochrome c oxidase subunit 2	P00403	MT-CO2	Oxidoreductasa
9. Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	P36957	DLST	Acetiltransferasa/ aciltransferasa
10. Hydroxyacylglutathione hydrolase, mitochondrial	Q16775	HAGH	Hidrolasa
11. Dynamin-like 120 kDa protein, mitochondrial	O60313	OPA1	Hidrolasa/ pequeña GTPasa
12. Single-stranded DNA-binding protein, mitochondrial	Q04837	SSBP1	Proteina unión al DNA
13. Transforming protein RhoA	P61586	RHOA	Pequeña GTPasa
14. Sideroflexin-1	Q9H9B4	SFXN1	Proteina acarreadora
15. NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 13	Q9P0J0	NDUFA13	Deshidrogenasa
16. NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial	P19404	NDUFV2	Deshidrogenasa/ Reductasa
17. Bis[5'-nucleosyl)-tetraphosphatase [asymmetrical]	P50583	NUDT2	Hidrolasa
18. ADP/ATP translocase 1	P12235	SLC25A4	Proteina acarreadora
19. Prohibitin	P35232	PHB	Desacetilación de histonas
20. Carnitine O-palmitoyltransferase 1, liver isoform	P50416	CPT1A	Acetiltransferasa/ aciltransferasa

Table 2 Quantitative Analysis Sub expressed polypeptides of underexpression identified in extracts of whole-brain proteins with Alzheimer's disease were identified by means of iTRAQ labeling and mass spectrometry.

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Effects of NBeylax functionalized TiO_2 nanoparticle administration on the DNA of cancer cells

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Abstract

Innovation in the area of nanotechnology is focusing on the development of therapeutic treatments that surpass traditional ones. NBeylax is a nanobiotech product with proven capabilities as a disinfectant and sanitizer, covering a wide spectrum of effectiveness against pathogens ranging from mycobacteria to viruses. In order to know if NBeylax is also effective against cancer cells and thus to assess its therapeutic potential against these diseases; The rat was used as an experimental study model to know the effects of the administration of NBeylax on this type of cells. The biocompatibility results showed that NBeylax has effects on the population of cancer cells and not on healthy tissue. It seems that these effects are due to the action of the NBeylax product on DNA since the studies of apoptosis demonstrate this.

Nanotechnology, biocompatibility, cancer, NBeylax, central nervous system, DNA, apoptosis

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Introduction

The great success of the "Nano Era" is the knowledge to handle the fundamental and molecular structure of matter, as well as its three-dimensional position. That is, to develop and / or modify the atomic or molecular architecture to create new materials for a specific application. One of the greatest advantages of these nanomaterials is the size of the particles, because with the "miniaturization" the contact surface is increased, and therefore the reactivity of the constituent elements, thus a substance that is inert in the Micro or macro scale has properties programmed at "nano" level (1-5). Gresmex has applied the development of this technology to solve problems related to health (6-11). This nanobio-molecule, whose registered trademark is Nbelyax®, with a patent application in Mexico and in more than 150 countries, has the capacity to act At the level of the genetic material.

How does it work? It functions as a bioselective catalyst programmed to inhibit the replication, transcription and translation of genetic material from pathogens.

Given the above, studies have been done on the ability of Nbelyax as a potential therapeutic nanofarmaco to treat diverse affections. Among them and the most interesting is cancer.

Thus, in order to demonstrate that Nbelyax can participate in the control of these cells through their selective mechanisms towards the genetic material such as DNA, it was decided to study the effects of Nbelyax® through in vivo studies on tissue Healthy and cancerous.

Therefore, in this work an experimental strategy was designed to test the effects of NBeylax on cancer cells, taking advantage of the effects it has on DNA.

Materials and methods

Animals

Ten male Sprague-Dawley rats, weighing approximately 250-300 g, were used in this study. The procedures were carried out in accordance with the Society of Neuroscience's policy on the use of animals in Neuroscience research.

Stereotactic surgery:

Stereotactic rat brain surgery. The animals were anesthetized with a combination of ketamine (100 mg / kg ip) and xylazine (10 mg / kg ip) and mounted in a stereotaxic frame (Kopf, Tujunga, CA) with body temperature maintained at 37 ° C Harvard Apparatus, Ealing, UK). After a midline incision, small holes were made in the skull at the coordinates 2.5 mm posterior to the bregma and 4.2 mm to the right and the left of the midline. A 25 gauge stainless steel cannula containing 2 ul of NBeylax was introduced 7.0 mm dorsally from the dura mater with the dorsal margin of the central tonsillar nucleus. The incision was then sutured, antibiotic and analgesic cream applied to the wound. The animals were then allowed 5 days of recovery, during which their behavior was observed to ensure that they were not in danger.

C6 cell tumors obtained from the American Tissue Culture Collection (Rockville, MD) under sterile conditions at 37 ° C and a humid atmosphere containing 5% CO were cultured. When a sufficient number of C6 cells were obtained, they were washed with a saline solution. Subsequently 1x107 C6 cells were inoculated intraperitoneally in a male Wistar rat to grow a tumor.

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After a 20-day growth period, the tumor had grown to an acceptable size. The tumor was surgically removed at 4 ° C and 1x107 cells were suspended in 500: 1 saline solution to be subsequently inoculated subcutaneously into the muscle of a group of Wistar rats (n = 100). The tumor developed in 80% of the animals. When the rats developed a 2 cm tumor (15 days after inoculation) they were separated into 2 groups as follows: (A) was control (no administration), (B) functionalized NBeylax was administered.

Histological studies

Nissl staining. - After sectioning, the cuts were immediately immersed in 2% TTC then in 0.9% NaCl at 37 ° C for 10 min for further use.

Staining with Hematoxylin and Eosin.- A hematoxylin and eosin staining was used to evaluate the integrity of the cells and the tissue, following the traditional protocols.

Cell Viability Test.- Trypan blue stain. Trypan blue is used as a marker of cell viability. Colorless living cells and dead cells are characterized by being tinted blue. Brain tissue sections were obtained from frozen sections made in a cryostat at -17 oC to 10 microns. Followed by a post-fixation with 4% PFA at -4o C. It was then incubated with trypan blue for 10 minutes and then rinsed with low concentrations of Et-OH.

Results

Effects of NBeylax's Brain Microinjection on Healthy Tissue

When we analyzed the effects of NBeylax on intact brain tissue, we observed that there are no visible effects on membrane integrity as well as cell appearance. Figure 1 shows hematoxylin-eosin photomicrographs of histological sections of rat brain tissue.

It also shows brain cuts treated with trypan blue where it was observed that they do not have the membrane involved which indicates that there is no apparent damage in the cell.

Brain tissue did not show evidence of inflammation or scarring near the path of microinjection and there were no morphological changes in neural cells.

Effects of microinjection of nanoparticles on cancer cells

The micrographs corresponding to this study are shown in figure 2 to corroborate that the damage suffered in the cancerous tissue was by the direct action of NBeylax on the DNA an experimental study of TUNEL was realized, which was positive for apoptosis in the tumor tissues Treated with NBeylax.

Discussion

In figure (1) it can be seen that the shape of the cell and the tissue of rat brains were normal compared to the microinjection of the same material into a cancerous tumor, figure (2) shows how the cell died by contact With NBeylax and its effects on DNA.

But why has lNBeylax differentiated the effects on healthy tissue and the cancerous tumor?

It may be because it might be related to the surface of the membrane, Thévenot et al (12) showed that some functionalized nanoparticles showed significantly greater toxicity in cancer cells than in control cell lines. In that study was shown by microscopic analysis the disruption of DNA which leads to cell death.

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Conclusions

In this study, a dramatic difference between the effects of NBeylax on healthy and cancerous tissue was observed.

Biocompatibility and no toxicological effects on healthy tissues were demonstrated when NBeylax was administered.

The selectivity of NBeylax to cancerous tissue and its effects on genetic material (DNA) was demonstrated by the results obtained through the use of the TUNEL technique.

Further experiments are needed to characterize the mechanism of action of this nanoparticle on DNA.

Annex

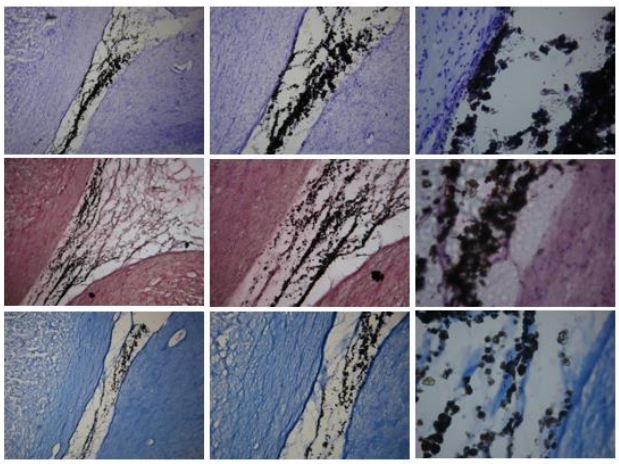

Figure 1 Hematoxylin-eosin micrographs of histological sections of rat brain tissue treated with NBeylax (A), (B) and (C), 5x, 10x and 40x haematoxylin-eosin staining. The same areas of the brain are shown in (E), (F) and (G), respectively, with trypan blue staining. The micrographs show intact cells in the treated area of the brain, with no evidence of inflammation or scarring in the injection trajectory area. There is no change in the shape of the cell membrane. Trypan blue staining demonstrated that microinjection of NBeylax does not have negative effects on the integrity of the cell membrane.

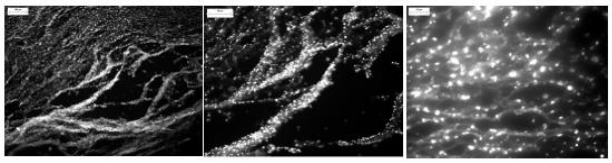

Figure 2 Micrographs of the TUNEL technique taken with fluorescence microscope (A) 5x (B) 10x and (40x) show affected cells in the treated area of the tumor.

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Article **ECORFAN Journal**
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Evaluation of anti-inflammatory, anti-nociceptive and toxicological effects of hydro-alcoholic extract of *C. pulverulentus* activity in a murine model

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Abstract

Costus pulverulentus is a plant used in traditional medicine for the treatment of infectious diseases and kidney inflammation. In order to check their antiinflammatory effect inflammation model carrageenan was used, the results showed a percent inhibition of edema at a concentration of 8 mg / kg similar to the effects of indomethacin (88.53 and 79.85%) EHCP while antinociceptive activity in the concentration of 8 mg / kg of EHCP exerted greater activity (57.58%) than that shown by aminopyrine in the late phase of the trial. Toxicological effects were determined by measuring the amount of digestive tissue ulceration, weight gain, behavioral changes, mortality and clinical signs and symptoms during administration of the treatment; treated groups EHCP concentrations or vehicle showed no apparent changes in the toxicological study period. This coupled with the anti-inflammatory and antinociceptive activity shown by the EHCP suggests that *C. pulverulentus* may be a desirable alternative to inflammatory conditions.

Ethnomedicine, carrageenan, side effects, *C. pulverulentus.*

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Introduction

The inflammation is a natural response of the organism to potentially harmful agents, under normal conditions this process is considered beneficial (Gupta et al., 2015, Lin et al., 2015), however, the excessive duration of this, caused by different Pathological conditions generate complications that can induce tissue loss (Komatsu et al., 2013). The drugs of choice to cope with this are non-steroidal anti-inflammatory drugs (NSAIDs), daily use of these and poor diet can lead to gastrointestinal diseases such as gastritis or stomach cancer (Salvatierra et al., 2006, Pérez et al. ., 2002). An alternative for the treatment of these diseases is usually the use of medicinal plants that apparently do not generate side effects, in Huasteca Potosina there is a wide range of medicinal plants, among them is Costus pulverulentus, belonging to the family of Costaceae, The main ethnomedical uses are in the treatment of renal, hypoglycemic, pain and inflammation diseases (Guzmán & Guerrero, 2009; Quintans et al., 2010). Therefore, the present research aimed to evaluate the antiinflammatory, antinociceptive and side effects of a hydroalcoholic extract of C. pulverulentus (EHCP) in order to show the ethnomedical uses referred to.

Methodology

Obtaining the extract

The plant material of C. pulverulentus was collected in the municipality of Aquismón (21 ° 37'5 "N 99 ° 01'51" O), S.L.P., Mexico in July 2015 in the rainy season.

The stems were washed and subjected to a drying process under dark conditions for one week, after finishing this process they were processed in a mechanical mill to obtain fine particles (Mahecha, 2007). To obtain the extract, the percolation technique was used, placing the plant material with 70% ethanol in a separating flask covered with aluminum to avoid exposure to light. The percolation was done by dripping and the extract obtained was evaporated to dryness (Carreón & García, 2010).

Experimental animals

For the determination of the anti-inflammatory and antinociceptive activity of the EHCP, female Wistar rats of 4 to 6 weeks of life with an average weight of 160-180 gr were used. Which were maintained in a control of temperature, humidity, free access to water and food with light / dark cycles of 12 hours each. The duration of the experiment was as short as possible, always considering that the number of animals used was the minimum necessary. Each animal was sacrificed following ethical guidelines for research on pain in experimental animals of the International Pain Association (Zimmerman, 1983).

In the toxicological evaluation 16 female Wistar rats of 4-6 weeks of age with a weight of 160 - 180 gr. Maintained under controlled temperature and humidity conditions with free access to water and food in 12-hour light / dark cycles. The animals were divided into 4 treated groups as follows: one control group with Indomethacin, one group with deionized water and two more with concentrations of 8 or 4 mg / kg EHCP.

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Model of Carrageenan Inflammation

The animals were divided into 4 groups composed of 4 rats each, which were pretreated with oral administration of EHCP at doses of 8 or 4 mg / kg, a positive control group, given Indomethacin (10 mg / kg) And a negative control group, to which only deionized water and DMSO (5: 1, vehicle) were given.

One hour after administering the treatments, inflammation was induced by injection of 1% carrageenan (Sigma, USA) into the sublantar region of the right paw of the animals (Winter et al., 1962).

The inflammatory process was measured with the aid of a digital plethysmometer (UGO BASILE), quantifying the volume of edema induced every hour for six hours. The percentage of the anti-inflammatory effect was calculated by the following equation:

$$\%\ \text{"Inhibition of edema"} = \frac{(\text{Ct-Co})\ \text{control} - (\text{Ct.-Co})\ \text{"Treated"} * 100}{(\text{Ct-Co})\ \text{control}} \quad (1)$$

Where Ct is the paw volume at time t after carrageenan injection and Co is normal leg volume before carrageenan injection.

Nociceptive model for formalin.

The animals were divided into 4 groups composed of 4 rats each, which were pretreated with oral administration of EHCP at a dose of 4 mg / kg body weight or 8 mg / kg, a positive control group administered aminopyrine (2 mg / kg) and a negative control group, to which only the vehicle was administered.

One hour after administration of the treatment, pain production was induced by 3% formalin (Sigma, USA) (Zimmerman, 1983) at doses of 20 μg per gram of weight in the subplantar region of the left paw of experimental animals.

The rats were placed in transparent cages for the purpose of observing their behavior. The time of licking and paw shaking was quantified for 30 minutes over 5 minute periods. The percentage of antinociception was calculated by the following equation (Isiordia, et al., 2010):

$$\%\ \text{antinocicepción} = \frac{\text{"Lamination T" "without" "Drug Lamination T-T} * 100\text{"}}{\text{"Lid Tide" "without" "drug"}} \quad (2)$$

Toxicological evaluation

During the treatment of the animals clinical signs and behavioral changes were recorded, increasing weight from day 1 to day 7.

At the end of the experiment the animals were sacrificed. A sagittal cut in the peritoneal area of the animals showed the presence of ulcerations in the gastrointestinal tract caused by the treatments. The number of ulcers was evaluated on a scale of 0 to 4 crosses, with one cross (0-2 ulcers), two crosses (3-5 ulcers), three crosses (6-10 ulcers) and four crosses (11-20 ulcers) (González & Rodríguez, 2014).

Statistic analysis

The normality criteria were reviewed in the generated data and later the ANOVA test was used to determine differences between the groups, taking as significant a value of $p < 0.05$.

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For the generation of graphs, we used the program Excel and Graph Pad Prism version 6.5.

Results

Figure 1 shows the results of anti-inflammatory activity, as shown, the Indomethacin-treated group had a significantly higher anti-inflammatory effect (84.53%, p <0.0032) than the concentrations of EHCP used during the first 2 hours of treatment. Both EHCP concentrations increase their constant anti-inflammatory effect from the third hour to the end of treatment, whereas the 8 mg / kg EHCP concentration exerts a similar effect to that of the control drug in the sixth hour (79.85%)..

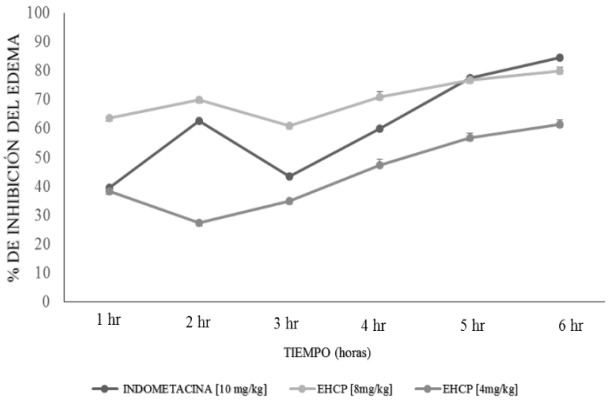

Figure 1 Anti-inflammatory effect of EHCP.

Pain is a symptom widely associated with inflammatory processes and is widely investigated to provide effective therapeutic solutions; The results obtained in the nociceptive model by formalin are shown in figure 2.

The early phase (0-5 minutes) is the result of a stimulus of nociceptive receptors caused by formalin, in this phase the drug aminopyrin showed a percentage of Antinociception of 24.57%, superior to those exerted by EHCP concentrations, although without significant difference between them (p> 0.05). In the late phase (15-30 minutes) the antinociceptive effect exerted the concentration of 8mg / kg of EHCP (57.58%) is higher compared to the antinociceptive effect of aminopyrin (45.89%) however the results do not show significant differences Among them (p> 0.05).

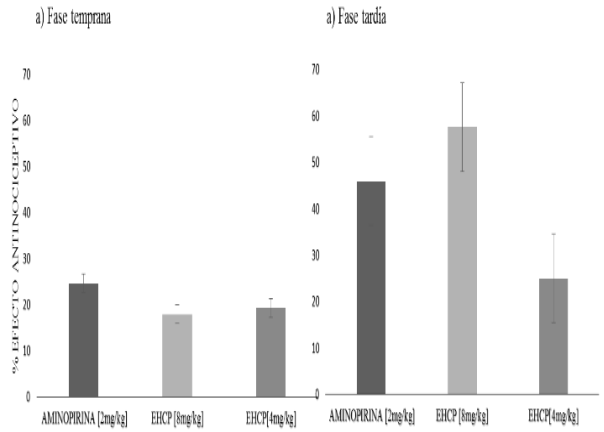

Figure 2 Inhibition of pain mediated by C. pulverulentus. A) Early phase, b) Late phase.

In order to show the possible adverse effects caused by treatments in animals, the number of ulcers present in the digestive tissue was quantified (figure 3).

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The control group treated with Indomethacin showed a total of 16 ulcers on average, significantly higher ($p < 0.0036$) than vehicle-treated groups (8 ulcers) or EHCP concentrations (8 and 9 ulcers respectively), which also showed Gastrointestinal damage but to a lesser extent than the Indomethacin control group, possibly due to the stress to which the animals were subjected during treatments.

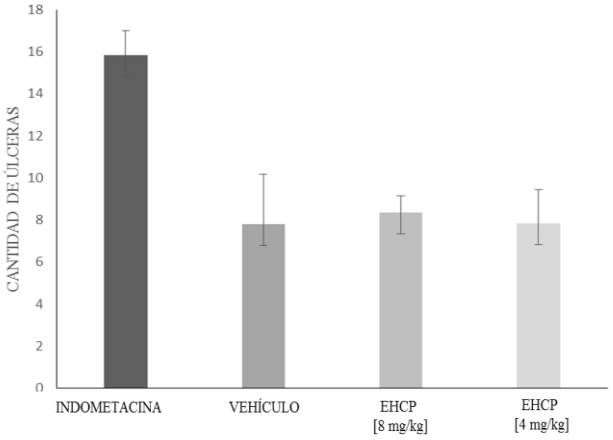

Figure 3 Number of ulcers observed in stomach and intestinal tissue of experimental animals in different groups.

The weight gain obtained for each treated group is shown in figure 4, it is observed that the groups treated with the vehicle or the concentrations of the EHCP maintain a constant weight gain while the group treated with Indomethacin maintains a significant weight gain ($P < 0.0001$) low compared to the other groups, possibly due to drug-induced ulceration.

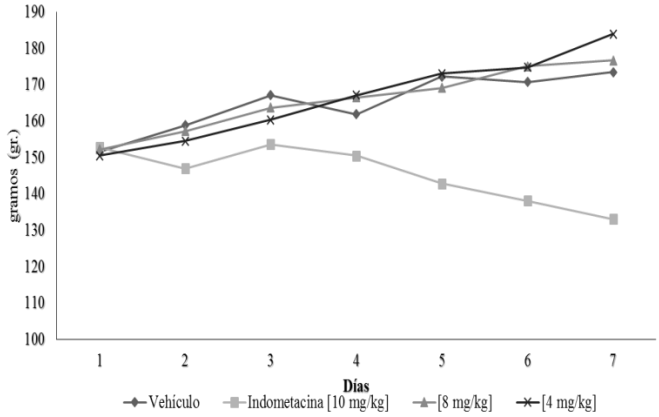

Figure 4 Weight gain in grams of the animals during the treatment period.

Changes in behavior, mortality, clinical signs and symptoms (Table 1) were observed during administration of the treatments. A significant change was observed in the animals treated with Indomethacin, which present aggression, polydipsia, sedation, and irritability until reaching the death of this group, vehicle-treated groups, and EHCP concentrations show no apparent changes.

		GRUPOS DE ESTUDIO			
Parámetros evaluados		Vehículo	Indometacina [10 mg/kg]	EHCP [10mg/kg]	EHCP [4mg/km]
Mortalidad		0/4	4/4	0/4	0/4
Alteraciones neurológicas	Inmovilidad	s/c	++	s/c	s/c
	Sedación	s/c	++	s/c	s/c
	Polidipsia	s/c	++	s/c	s/c
	Agresividad	s/c	++	s/c	s/c
Anormalidad del comportamiento	Irritabilidad	s/c	++	s/c	s/c
	Pasividad	s/c	+	s/c	s/c
Condiciones de piel y mucosas	Piel	s/c	s/c	s/c	s/c
	Mucosas	s/c	s/c	s/c	s/c
	Heces	s/c	consistencia acuosa	s/c	s/c
(S/C) Sin cambios, (+) Cambio leve, (++) Cambio moderado.					

Table 1 Signs and symptoms observed during treatment.

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Conclusions

The EHCP shows anti-inflammatory and antinociceptive effects similar to the drugs used as control, without provoking apparent side effects, so it could be considered as an effective treatment alternative whenever more in-depth studies corroborating the safety of its use by time prolonged.

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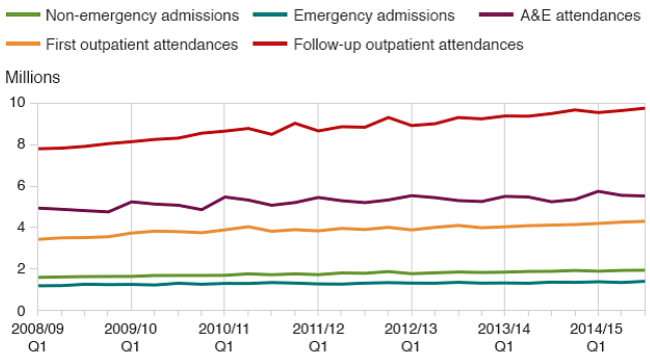

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