ABSTRACT

Background. *Encelia canescens* Lam is a plant traditionally used in Peru for medicinal purposes, and is attributed antioxidant properties, indicating that it could be used in the prevention of non-communicable diseases. **Objective:** This study aims to evaluate the protection of erythrocytes from lipoperoxidation and the anti-inflammatory effect of ethanolic extract of *E. canescens* in mice. **Materials and methods:** Protection from lipoperoxidation was evaluated by inhibition of hemolysis and quantifying malondialdehyde (MDA) concentration against oxidative stress induced with hydrogen peroxide (H₂O₂) at 200, 150, 100, 50 and 25 μg/mL. *E. canescens* concentrations. The 1% carrageenan-induced air pouch model was used for evaluated inflammation, where albumin, total proteins, MDA, number and leukocyte differentiation were determined in the exudate, and a histopathological evaluation was performed. The concentrations evaluated were 100, 250 and 500 mg/kg of *E. canescens*.

**Results:** All the concentrations evaluated protected erythrocytes from lipoperoxidation (p<0.05), being E.D. value 200 μg/mL. Regarding anti-inflammatory effect, the albumin, total proteins and MDA values of the treatment groups were lower than carrageenan 1% group (p<0.05), but, due to less leukocyte migration and presence of macrophages and the histopathological evaluation, the E.D value was 500 mg/kg. **Conclusion:** Ethanolic extracts of *E. canescens* leaves protect erythrocytes from lipoperoxidation and have dose-dependent anti-inflammatory effects maybe for presence of p-hydroxyacetophenone-derived, and these could be new safer anti-inflammatories.

**Key words:** *Encelia canescens* Lam, Hemolysis, Malondialdehyde, Lipoperoxidation, Anti-inflammatory, Carrageenan.

INTRODUCTION

Noncommunicable diseases (NCDs) are a health problem for years in low and medium-income countries, generating a negative impact on social and economic development worldwide, being the inflammatory processes one of the ones that demand the highest costs worldwide, without having the treatment it can lead to much more serious problems. The four main types of NCDs are cardiovascular, cancer, lung, and diabetes; in such a way that one of the main causes of these diseases is oxidative stress, a process that shows an imbalance between oxidants and antioxidants and that is the cause of cellular damage.

Peru is considered an area of high plant richness and mainly of the Asteraceae, with more than 1613 species within them we have the species *Encelia canescens* Lam (*E. canescens*) known as “coronilla del fraile” or “herba lingo” that is empirically used for its analgesic,11 galactophoric properties,12 menstruation regulation,13 and cancer treatment.13-15 Therefore, protection from lipoperoxidation through antioxidants application, particularly antioxidants derived from medicinal plants, may be a possible strategy to protect cellular damage generated by oxidative stress.16,17

This study therefore aimed at evaluating protection from lipoperoxidation in erythrocytes and the anti-inflammatory effect of *E. canescens* leaves ethanolic extract in mice.

MATERIALS AND METHODS

The study was conducted at the Pharmaceutical Research Center of the Faculty of Pharmacy and Biochemistry at Norbert Wiener University.

**Plant material and extract**

Plant species was collected in the province of Huara, North of Lima to 250 m.s.n.m. The taxonomic and anti-inflammatory activity16,18, but triterpenoids has same anti-inflammatory activities.19-21

Antioxidant capacity can be measured in lipoperoxidation processes and also by means of oxidative stress markers; since lipid oxidation has been shown to be associated with damage at level of the cell membrane, this leads to apoptosis in a wide variety of cells.16,17 Therefore, protection from lipoperoxidation through antioxidants application, particularly antioxidants derived from medicinal plants, may be a possible strategy to protect cellular damage generated by oxidative stress.16,17

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**E-mail:** juana.chavez@uwiener.edu.pe

**Facultad de Farmacia y Bioquímica, Universidad Norbert Wiener, PERÚ.**

**Facultad de Farmacia y Bioquímica, Universidad Nacional de Trujillo, PERÚ.**

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classification of the species was carried out at the Museo de Historia Natural of Universidad Nacional Mayor de San Marcos (Nº 198-USM-2018). The process of obtaining the ethanolic extract from the *E. canescens* leaves was carried out in accordance with the stipulations of Lock O. (2016).\(^{21}\)

**Phytochemistry analysis**

The process of phytochemistry analysis of ethanolic extract from the *E. canescens* leaves was carried out in accordance with coloring and precipitation techniques of Lock O. (2016).\(^{21}\)

**Biological material**

For the evaluation of oxidative stress protection, a 2 mL sample of erythrocytes was obtained from rats of the Holtzman strain of 12 weeks of age weighing 220 ± 10 g from Instituto Nacional de Salud (INS) and to evaluate the anti-inflammatory effect, male mice 25 g each, strain Balb/C53/CNPB was used. This study was approved by the Norbert Wiener University Ethics Committee under opinion N° 005-08-2019 FB/UPNW.

**Hemolysis and malondialdehyde quantification**

Erythrocytes protection from lipoperoxidation induced by H\(_2\)O\(_2\) was evaluated by determining hemolysis and quantifying malondialdehyde (MDA) following the methods of Fátima et al. (2013) and Esterbauer et al. (1990) evaluating concentrations of 200, 100, 50 and 25 µg/mL of *E. canescens*.\(^{23,24}\)

**Anti-inflammatory effect**

1% carrageenan-induced air pouch model described by Duarte et al. (2016) was used, the inhibition of inflammation was measured with albumin, total protein and MDA parameters at concentrations of 100, 250 and 500 mg/ kg of *E. canescens*.\(^{25}\) Number and differentiation of leukocytes obtained from the exudate was determined by Wright staining as indicated by Mahat et al. (2010) with some modifications.\(^{25}\)

**RESULTS**

*E. canescens* ethanolic extract was soluble in polar solvents and insoluble in apolar solvents. In addition, the presence of secondary metabolites such as flavonoids, phenolic compounds, and alkaloids was identified by qualitative analysis.

In the evaluation of erythrocyte protection against induced oxidative stress via H\(_2\)O\(_2\), inhibition of hemolysis and release of MDA content depend on the concentration of the extracts, so concentration of 200 µg/mL presented highest protection (Tables 1 and 2 and Figure 1).

**DISCUSSION**

Secondary metabolites such as flavonoids, alkaloids, tannins, steroids and/or triterpenes were identified in this study. These metabolites have also been identified in Chile by Cayún et al. (2015), except for alkaloids; it seems that the climate and altitudinal floors in Peru are favorable for the development of these secondary metabolites.\(^{13,28}\) According to the reviewed literature, flavonoids, tannins, coumarins, steroids and/or
triterpenes are metabolites that have demonstrated anti-inflammatory and antioxidant effects, contributing to the prevention of chronic diseases of higher prevalence.29,30

In Table 2, *E. canescens* 200 µg/mL presented greater protection against hemolysis compared to the H₂O₂ control group (p < 0.05) at the three-time marks (30, 60 and 90 min). Radical H₂O₂ oxidizes the ferrous ion of hemoglobin to ferric, producing methemoglobin in the Fenton reaction, generating hydroxyl ion (•OH)31, a reactive species capable of damaging DNA, lipids, and cell proteins, among others.32 Secondary metabolites with antioxidant properties transfer electrons to the hydroxyl ion causing reduction of the ferric ion and stabilizing hemoglobin, preventing hemolysis and restoring the balance of the plasma membrane in erythrocytes.33

In Figure 1, the concentration of MDA in erythrocytes is shown after having been exposed to oxidative stress with H₂O₂; concentration-dependent MDA decrease of *E. canescens* extracts is evidenced, showing a significant difference with the H₂O₂ group, the one in which the concentration of 200 µg/mL stands out. Lipoperoxidation occurs in the erythrocyte membrane and MDA is an indicator of this phenomenon, which is mediated by inflammatory processes that will damage cellular components as well as increased levels of hydroxyl radical (•OH), superoxide (O₂•⁻), radicals derived from nitrogen (NO) which destabilize the cell membrane.34-36 In this sense, *E. canescens* extract stabilizes the radicals that will cause lipoperoxidation as it is an electron donating compound.

Table 3: Effect of *E. canescens* on biochemical parameters in air pouch exudate after administration of carrageenan at 1%.

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Albumin (g/dL)</th>
<th>Total proteins (g/dL)</th>
<th>MDA (nmol/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observer</td>
<td>0.26ª</td>
<td>2.13ª</td>
<td>1.12ª</td>
</tr>
<tr>
<td>Control</td>
<td>1.84</td>
<td>5.31</td>
<td>1.67</td>
</tr>
<tr>
<td>Dexamethasone 2 mg/kg</td>
<td>1.21ª</td>
<td>3.57ª</td>
<td>1.44ª</td>
</tr>
<tr>
<td><em>E. canescens</em> 100 mg/kg</td>
<td>1.65ª</td>
<td>4.58ª</td>
<td>1.57ª</td>
</tr>
<tr>
<td><em>E. canescens</em> 250 mg/kg</td>
<td>1.48ª</td>
<td>4.15ª</td>
<td>1.45ª</td>
</tr>
<tr>
<td><em>E. canescens</em> 500 mg/kg</td>
<td>1.01ª</td>
<td>3.67ª</td>
<td>1.24ª</td>
</tr>
</tbody>
</table>

Table 4: Effect of *E. canescens* on leukocyte infiltration in air pouch exudate after administration of carrageenan at 1%.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leukocytes x mm³</th>
<th>Leukocyte differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Polymorphonuclear x 10⁶ / mm³</td>
<td>Monocytes x 10⁶ / mm³</td>
</tr>
<tr>
<td>Observer</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>24.16</td>
<td>15.56</td>
</tr>
<tr>
<td>Dexamethasone 2 mg/kg</td>
<td>4.15ª</td>
<td>2.81ª</td>
</tr>
<tr>
<td><em>E. canescens</em> 500 mg/kg</td>
<td>4.57ª</td>
<td>2.80ª</td>
</tr>
<tr>
<td><em>E. canescens</em> 250 mg/kg</td>
<td>6.98</td>
<td>4.08</td>
</tr>
<tr>
<td><em>E. canescens</em> 100 mg/kg</td>
<td>8.38</td>
<td>4.14</td>
</tr>
</tbody>
</table>
inhibiting the lipoxygenase pathway 5 which prevents the inflammation cascade from forming and prevents the release of inflammation mediators. 37, 38 Furthermore, steroids participate by inhibiting the inflammatory process, since they stabilize the lysosome membrane, intracellular particles that contain proteases and hydrolytic enzymes which form inflammation chemical mediators, preventing the disintegration of said lysosomes that are produced in inflammatory processes and are responsible for damage and other inflammatory phenomena. 39 When evaluating the biochemical parameters, E. canescens 500 mg/kg presented better results, showing that the higher the concentration, greater anti-inflammatory effect. Cochachi and Fernández (2015), had similar results when evaluating the ethanolic extract of the stems of E. canescens 40 where they found a total proteins and albumin decrease, being attenuated after the administration of the treatment groups, being attenuated after the administration of the treatment groups, being most activity concentration was 400 mg/kg was related to the lower leukocyte migration in the exudate and cell infiltration. The anti-inflammatory potency of the extract 500 mg/kg significantly reduced inflammatory changes with less edema and cell infiltration. The anti-inflammatory potency of the extract 500 mg/kg was related to the lower leukocyte migration in the exudate and cell infiltration. The anti-inflammatory potency of the extract 500 mg/kg was related to the lower leukocyte migration in the exudate and cell infiltration. The anti-inflammatory potency of the extract 500 mg/kg was related to the lower leukocyte migration in the exudate and cell infiltration. The anti-inflammatory potency of the extract 500 mg/kg was related to the lower leukocyte migration in the exudate and cell infiltration.

CONCLUSION
Under experimental conditions, the ethanolic extract of Encelia canescens Lam leaves protects erythrocytes from lipoperoxidation induced via H2O2 and presents an anti-inflammatory effect at dose-dependent in carrageenan 1%-induced airbag model, maybe for presence of p-hydroxyacetophenone-derived compounds probably be related to anti-inflammatory activity by being closely related to cyclooxygenase inhibitors structure. 14, 43 Anti-inflammatory activity by others acetophenone derivative had be reported. 44-46

The anti-inflammatory effect of E. canescens was examined histologically (Figure 2), in the normal tissue (observer) inflammation was not evidenced where a layer of flattened cells superimposed on loose vascular connective tissue is observed. In carrageenan 1% group, macrophages and fibroblasts increased in the lining layer of flattened cells in the groups, observing inflammatory changes such as leukocyte infiltration and edema (evidenced by tissue thickening). E. canescens 500 mg/kg significantly reduced inflammatory changes with less edema and cell infiltration. The anti-inflammatory potency of the extract 500 mg/kg was related to the lower leukocyte migration in the exudate and the lower concentration of MDA.

Although the relationship between chemical composition and activity has not been established, some reported compounds may be responsible for the activity. Bohlmann et al (1982) reported presence aromatic compounds like p-hydroxyacetophenone derivatives, p-hydroxyacetophenone-derived compounds probably be related to anti-inflammatory activity by being closely related to cyclooxygenase inhibitors structure. 14, 43 Anti-inflammatory activity by others acetophenones derivative had be reported. 44-46

CONFLICTS OF INTEREST
The authors declare no conflicts of interest in this study.
Fernández-Flores N et al.: Protection of Erythrocytes against Lipid Peroxidation and Anti-inflammatory Effects of Ethanolic Extract of Encelia canescens Lam Leaves in Mice


GRAPHICAL ABSTRACT

ABOUT AUTHORS

Fernández-Flores Nélber. Student of Pharmacy and Biochemistry at Universidad Norbert Wiener. He is part of merit school for being awarded the “Alcibiades Horna Figueroa” scholarship. Researcher at Centro de Investigación Farmacéutica of the same university, Asociación Peruana de Estudiantes de Farmacia y Bioquímica (APEFYB). He has been part of the research seedbed group since 2017. He participated as an assistant and speaker at Congreso Nacional Peruano de Estudiantes de Farmacia y Bioquímica (CONAPEFYB), scientific meetings and institutional symposiums obtaining different recognitions. He is currently practicing in pharmaceutical care.

Rojas-Cardenas Nathalie F. She has been part of the research seedbed group of Universidad Norbert Wiener since 2017. Researcher at the Pharmaceutical Research Center of the same university. She participated as an assistant and speaker at Congreso Nacional Peruano de Estudiantes de Farmacia y Bioquímica (CONAPEFYB), scientific meetings and institutional symposia obtaining different recognitions. She currently works in pharmaceutical industry.

Vásquez-Quispe Ángel D. He has been part of the research seedbed group of Universidad Norbert Wiener since 2017. Researcher at the Pharmaceutical Research Center of the same university. He participated as an assistant and speaker at Congreso Nacional Peruano de Estudiantes de Farmacia y Bioquímica (CONAPEFYB), scientific meetings and institutional symposia obtaining different recognitions. He currently works as a clinical pharmacy assistant.

Chávez-Flores Juana E. Doctor in Pharmacy and Biochemistry (2011) and Master in Experimental Pharmacology (2007), both of Universidad Nacional Mayor de San Marcos. Pharmacist by Universidad Norbert Wiener (2004), she did an international internship (2020) at the Laboratory of Cellular Biology and Genotoxicology - Universidad Técnica Particular de Loja - Ecuador, participates in phytochemical, pharmacological and toxicological research projects, teacher and researcher, co-organizer of ECI: International Scientific Meeting, member of the "Centro Peruano de Investigación y Desarrollo de Recursos Naturales en Salud INKA HAMPI".
Justil-Guerrero Hugo J. He is professor and researcher in Faculty of Pharmacy, Universidad Norbert Wiener, Lima, Peru. He researches about products with anti-inflammatory properties and has experience on use of animal models to discover new potential drugs related to inflammatory diseases.

Parreño-Tipian Juan M. Professor and researcher in Faculty of Pharmacy by Universidad Norbert Wiener and Universidad Nacional Mayor de San Marcos. Pharmaceutical specialist in biochemical análsis. He Has a degree in education, and he is doctor in Pharmacy and Biochemistry and other in Biochemistry and Nutrition. He has a diploma in Clinical Pharmacy and Pharmaceutical Care. He is a member of the Faculty of Pharmacy and Biochemistry Council - UNMSM and a member of the Chemical Society of Peru. Researcher of the ANBICLIT Research Group (UNMSM)

Silva–Correa Carmen Rosa. Professor in the Department of Pharmacology of the Universidad Nacional de Trujillo holds a degree in Pharmacy and Biochemistry (2011), Master of Chemical Sciences (2017), graduate student at Doctoral program in Biomedical Sciences since 2019. Currently participates in research projects aimed at the phytochemical characterization of medicinal plants, focusing on antimalarial and leishmanicidal activity. In addition, she participates in the evaluation of the wound healing activity of traditional medicinal plants from Peru.

Villarreal–La Torre Víctor E. Master of Chemical Sciences, holds a degree in Pharmacy from Universidad Nacional de Trujillo (2011). Professor in the Medicinal Chemistry undergraduate program and the Molecular basis of the Action of Xenobiotics postgraduate program at the Universidad Nacional de Trujillo. He currently executes research projects aimed at the discovery of antimicrobial compounds in medicinal plants. Graduate student at Doctoral program in Pharmacy and Biochemistry since 2019.