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Trabajo Científico

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Actividad antiinflamatoria de los extractos acuoso y metanólico de Oenothera rosea L’ Hér. ex Ait en la rata

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Abstract

Oenothera rosea L’ Hér. ex Ait. (Onagraceae) is commonly known as hierba del golpe. It is used in Mexican Folk Medicine to treat inflammatory, renal and bacterial diseases. The aim of this study was to evaluate the anti-inflammatory activity of aqueous and methanolic extracts of this plant. Aqueous extract at a dose of 500 mg/kg body weight (b.wt.) and methanolic extract (100 mg/kg b.wt.) of Oenothera rosea were evaluated for anti-inflammatory activity using the cotton pellet-induced granuloma formation model in rat and histological techniques. Both extracts produced a significant decrease of the inflammatory process in relation with the control groups (p<0.05). The anti-inflammatory effect of methanolic extract was similar to the effect of indomethacin. This data was supported by the histological results. No extracts produced gastrointestinal damage. The LD$_{50}$ of aqueous and methanol extracts, was higher than 40 and 8 g/kg, respectively. These results suggest that both extracts of Oenothera rosea induced anti-inflammatory activity and it was considered not toxic.

Resumen

Oenothera rosea L’ Hér. ex Ait. (Onagraceae) es comúnmente conocida como hierba del golpe y utilizada en la medicina Tradicional Mexicana para el tratamiento de padecimientos inflamatorios, renales y bacterianos. El objetivo de este estudio fue evaluar la actividad antiinflamatoria de los extractos acuoso y metanólico de esta planta. Los extractos acuoso (500 mg/kg de peso corporal (p.c.)) y metanólico (100 mg/kg p.c.) de Oenothera rosea fueron evaluados mediante el modelo del granuloma en ratas y técnicas histológicas. Ambos extractos indujeron una disminución significativa del proceso inflamatorio en relación con los grupos testigo (p<0.05). El efecto antiinflamatorio del extracto metanólico fue similar al efecto de indometacina. Este dato fue corroborado por los resultados histológicos. Ningún extracto produjo daño gastrointestinal. La DL$_{50}$ de los extractos acuoso y metanol, fue mayor de 40 y 8 g/kg, respectivamente. Estos resultados sugieren que ambos extractos de Oenothera rosea produjeron actividad antiinflamatoria y fueron considerados no tóxicos.

Keywords: Oenothera rosea, Onagraceae, anti-inflammatory, cotton pellet granuloma, indomethacin

Palabras clave: Oenothera rosea, Onagraceae, antiinflamatorio, modelo del granuloma, indometacina

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Introduction

The use of plants with therapeutic effects is a common practice among the Mexican population. There are a great number of species with anti-inflammatory effects, among them is *Oenothera rosea* L’Hér. ex Ait (syn. *Hartmannia rosea* G. Don, *Oenothera purpurea* Lam., *Oenothera rubra* Cav., *Oenothera virgata* Ruiz y Pavón, *Hartmania virgata* Spach). It is commonly known as hierba del golpe, and the whole plant possesses medicinal properties useful in the treatment of skin diseases, renal and inflammatory diseases, hepatic pain, liver and skin problems, as well as anti-diarrheic effect. In Folk Medicine the whole plant is often used as an infusion for internal inflammation or as a cataplasm in topic swellings.

In spite of the traditional use of this plant, only a few detailed chemical and pharmacological studies that could support the anti-inflammatory activity have been reported. Meckes et al. performed a preliminary screening of the effect produced by methanolic extract of *Oenothera rosea* on carrageenan-induced rat paw edema, finding a higher anti-inflammatory effect of this extract during the 7 hours after the treatment, that is why these authors proposed this plant as a good candidate to be submitted for further investigation.

Due to its popular use in several diseases, in this study, the anti-inflammatory activity of aqueous and methanolic extracts of *Oenothera rosea* was evaluated. Besides the phytochemical and histological analysis and the acute toxicity were determined.

Materials and methods

Plant material

*Oenothera rosea* L’Hér. ex Ait, “hierba del golpe” (Onagraceae) was collected in August 2004 (Mexico City) and botanically authenticated by Biologist Laura Doval Ugaldé. The specimen was compared with a voucher specimen deposited in the herbarium of the National School of Biological Sciences, IPN (M. J. Díaz No. 164). The whole plant was shade-dried and powdered.

Preparation of the aqueous and methanolic extracts

During the experiments, the aqueous extract was prepared as an infusion every day (250 mg and 500 mg/mL water), just as the Mexican population has reported its use.

The methanolic extract was prepared with 200g of the dried powdered material, which was extracted by reflux with 2.0 L of methanol (analytical grade) for 9 hours. The dissolvent was replaced every three hours.

The extract was filtered and evaporated in a carefully regulated water bath that was maintained at 45°C until yielding a brown syrup extract weighing 18.5g (yield: 11.56%). A preliminary test for metabolites qualitative detection of both extracts were made.

Animals

Adult female Wistar rats and male NIH mice were used for this study. They were housed and maintained in the animal house at room temperature (22-24°C) and 50-55% relative humidity, with day/night cycles of 12 x 12h. They were fed with standard rodent diet and water *ad libitum*. Care and handling of the animals were in agreement with internationally accepted procedures and approved by our institutional committee following the recommendation included in the Mexican Technical Specifications for the Production, Care, and Use of Laboratory Animals.

Anti-inflammatory activity

Cotton pellet-induced granuloma formation in rats

A sterilized cotton pellet granuloma weighing 50 ± 5 mg was introduced subcutaneously, under sterile conditions into axilla, in an anaesthetized rat. The test groups of animals were treated orally with 250 or 500 mg/kg of the aqueous extract and with 100 mg/kg of the methanolic extract, daily for 7 days. Animals, in the control and reference groups, received 5% sodium bicarbonate solution, peanut oil and indomethacin (5 mg/kg in 5% sodium bicarbonate solution).

The animals were sacrificed on the eighth day. The pellets surrounded by granuloma tissue were dissected out carefully and recorded for wet weight, then, the pellets were dried at 60°C to a constant weight. The difference between the final weight of the pellet after drying and its initial weight was taken as the final granuloma weight.

Mean weight, of the granuloma tissue formed in each group, was obtained and the results were expressed in wet, dry and final granuloma weight.

Histological analysis

After dissection of granuloma tissue, a sample of tissue in touch with the granuloma, was fixed in 10% formaldehyde, during 48 hours, and then included in paraffin.

Later cuts of a 6 µm thickness of were carried out, which were mounted in a microscope slide and dyed with Hematoxilin-Eosin (topographic technique), toluidine blue for metacromasia, which allowed the demonstrate the presence of mast cells and with Mallory identify collagen fibers.

Gastroduodenal damage study

The abdomens of the rats from the cotton pellet-induced granuloma formation were opened. The stomach and duodenum were removed and opened then the tissue was examined for macroscopic lesions. The results were expressed as presence...
or absence of gastroduodenal irritation (redness and/or presence of ulcers).

**Acute toxicity test**
Acute toxicity study was performed in 48 male NIH mice weighing 28 ± 5 g, which were divided into 12 groups. Four animals were used for complete evaluation in each dosage level. Aqueous extract of *Oenothera rosea* at doses from 5 to 40 g/kg were administrated to the first five groups, methanolic extract at doses from 1 to 8 g/kg were administrated to the other five groups. Water and peanut oil, respectively, were administrated to both remaining groups (control groups). The animals had access to food and water *ad libitum*. During the first 6 hours, any motor and respiratory activity change, as well as any different sign from control group were observed and recorded, after the administration of the aqueous and methanolic extracts. For the next 7 days the number of dead animals was also recorded.

**Statistical analysis**
The data are presented as mean ± S.E.M and the statistical significance between groups was analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni’s test. The differences between groups were regarded as significant at p<0.05.

**Drug and chemicals**
Indomethacin was purchased from Sigma Chemical Co (St Louis, MO). Sodium bicarbonate was Baker and peanut oil (PO), A. R. (Mexico). All other reagents were analytical grade.

**Results and discussion**
The preliminary test showed the presence of alkaloids, reducing sugars, tannins, coumarins, flavonoids and cardiotonic glycosides.

In relation to the pharmacological activity, cotton pellet-induced granuloma formation was induced in rat model. This model has been widely used to assess the transudative, exudative and a proliferative phase of inflammation, due to the formation of granuloma tissue. This is a typical feature of subacute or chronic inflammatory reaction. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma, whereas the dry weight correlates well with the amount of granulomatous tissue formed14, 15, 16.

As shown in Figure 1, aqueous extract at doses of 250 and 500 mg/kg decreased the wet, dry and final granuloma weight. However, only at dose of 500 mg/kg the difference between test group and control group was statistically significant (p<0.05) in all cases.

![Figure 1. Anti-inflammatory effect of *Oenothera rosea* L. Hér. ex Ait extracts on final granuloma weight. Data represent mean ± S.E.M. *Significantly different from control p<0.05.](image)

The methanolic extract showed a high anti-inflammatory effect in final granuloma weight, being this result very similar to the one obtained with indomethacin (Figure 1).

The anti-inflammatory effect of some species has been attributed to the presence of several secondary metabolites like the flavonoids, which have the aptitude to modify eicosanoid biosynthesis (antiprostanoid and anti-inflammatory responses) as well as prevent the plaquet aggregation17, 18. Due to the fact that this class of metabolites was present in *Oenothera rosea* extracts, it is suggested that the same ones should be responsible for the anti-inflammatory activity of this plant.

Other metabolites classes with anti-inflammatory properties are the alkaloids and coumarins, which also were present in *Oenothera rosea* extracts. Alkaloids as the 5′-Hydroxymethyl-1′-(1,2,3,9-tetrahydro-pyrrolo [2,1-b] quinazolin-1-yl)-heptan-1-one isolated from *Sida cordifolia* Linn.19, the eleagnine obtained from the *Chrysothyllum albium* seeds20 and some coumarins as the umbelliprenin, distributed in the plants of Apiaceae family21 have been described as anti-inflammatory compounds.

Another possible mechanism of the anti-inflammatory effect of this plant is related to the presence of essential fatty acids in some species of the Onagraceae family, like *Oenothera biennis*, of which, the seeds are used to obtain the oil known as “evening primrose oil”, used like anti-inflammatory, due to its high indexes of linolenic acid (71.5 %) and γ-linolenic acid (7 - 14 %)22,23. This characteristic has caused that the evening primrose oil has being used mainly in circulatory, genital and dermatological disorders and rheumatism24.
Knorr and Hamburger\textsuperscript{25}, reported that the evening primrose oil contains lipophilic triterpenoids ethers as 3-O-trans-cafeol, which present anti-inflammatory properties.

The nonsteroidal antiinflammatory drugs (NSAID), like indomethacin, act on the active site of two cyclooxygenase isoforms: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), inhibiting prostaglandin biosynthesis. The gastrointestinal damage induced by these drugs is not selective, they inhibit the COX-2 isoform present in damaged or inflamed tissues and the COX-1, the protective isoform. When COX-1 is inhibited, gastrointestinal toxicity could present\textsuperscript{26, 27, 28}.

The results demonstrated that the groups treated with the aqueous and methanolic extracts, as well as the control groups, did not produce gastroduodenal irritation. Possibly, this specie has an anti-inflammatory mechanism COX-2 selective\textsuperscript{29}.

The macroscopic analysis shown that the stomach and duodenum of the groups treated with 5\% sodium bicarbonate solution and peanut oil presented a normal epithelium; in comparison with the group treated with indomethacin, in which the presence of irritation was observed along the duodenum and in some of the cases gastrointestinal sores were present. In all the groups treated with the aqueous and methanolic extracts of \textit{Oenothera rosea}, there were no signs of irritation or ulceration in the gastroduodenal tract.

Also, group treated with indomethacin, a very thin capsule was observed and in other occasions it was strongly adhered to the granuloma, unlike the groups treated with the aqueous and methanolic extracts in which the thickness of the capsule was bigger and an increase in the exudation of liquid was observed. In the groups treated with 5\% sodium bicarbonate solution and peanut oil, the thickest capsules were observed.

In the group treated with the aqueous extract (500 mg/kg), methanolic extract (100 mg/kg) and indomethacin (5 mg/kg) a decrease in the number of conjunctive tissue dispersed leukocytes was observed in comparison to the inside and adhered blood vessels ones. This change in the leukocytary infiltration is a defense mechanism, due to the fact that the leukocytes consume the aggressor agents, they destroy bacteria and other microorganisms, however their leucocytary products can also

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**Figure 2.** Tissue in contact with granuloma of a rat treated with indomethacin (5 mg/kg) (A), aqueous extract of \textit{Oenothera rosea} (500 mg/kg) (B), 5\% sodium bicarbonate (C), methanolic extract of \textit{Oenothera rosea} (100 mg/kg) (D). Cells presence: a) neutrophil, b) blood vessel, c) lymphocyte, d) mast cells with degranulation activity, e) mast cell without degranulation activity. Hematoxilin-Eosine technique 40X (A), 100X (B). Toluidine blue technique 40X (C), 100X (D).
induce tissue damage and increase the inflammation period. In the groups treated with 5% sodium bicarbonate and aqueous extract (500 mg/kg) a great number of mast cells in degranulation phase, were observed, possibly these treatments are favoring the mediators liberation as histamine, neutrophilic chemotactic factor and vasoactive intestinal peptide from this kind of cells that are carrying out the inflammatory process. The group treated with indomethacin, the anti-inflammatory reference drug and methanolic extract, decrease the degranulation activity, which allows to suppose that this ones are producing a cellular level recovery, diminishing the inflammatory process.

In all cases a great quantity of collagen fibers, as mechanism of tissue repair, was observed, principally for fibrosis, due to the persistence of the initial stimulus.

None of the doses of aqueous or methanolic extract produced mortality or any behavioral disorders.

Conclusions

The results of this study induced the anti-inflammatory activity of aqueous (500 mg/kg) and methanolic (100 mg/kg) extracts of Oenothera rosea, with no toxic effects at the administrated doses.

References


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