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Gastroprotective activity of *Hamelia patens* Jacq. in the ethanol-induced gastric injury model

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— Abstract—

Peptic ulcers affect about 10 % of the population worldwide and in some cases lead to the development of peritonitis, which can cause death. Since the diverse drugs used for the treatment of peptic ulcers all have serious adverse effects, it is necessary to seek new therapies. The scientific study of medicinal plants is important because they represent an important source for obtaining new drugs. *Hamelia patens* is utilized empirically to treat gastric ulcers in Mexico, but this practice is not based on a scientific foundation. The current contribution aimed to examine the possible gastroprotective effect of *Hamelia patens* with the model ethanol-induced gastric lesions in Wistar rats. The leaves of *Hamelia patens* were macerated to prepare the hexane, dichloromethane, and methanol extracts, which were administered orally at doses of 10, 30, and 100 mg/kg. Carbenoxolone (the reference drug) was given to the animals at 3-100 mg/kg. The most active extract was separated by column chromatography and the four fractions obtained were evaluated on the rats at 100 mg/kg. Whereas the dichloromethane and methanol extracts were active at all three doses tested, the hexane extract only showed gastroprotective activity at the highest dose (100 mg/kg). At 10 mg/kg, the dichloromethane extract was more effective than carbenoxolone at the same dose ($p<0.05$). Three of the four fractions of dichloromethane extract displayed gastroprotective activity ($p<0.05$), with ulcer index values of 42, 37, and 55 mm^2. In conclusion, *Hamelia patens* demonstrated gastroprotective activity in Wistar rats with ethanol-induced lesions and proved to have more than one active compound.

Keywords:

Medicinal plants; Hamelia patens; gastroprotection; gastric ulcers.

A peptic ulcer is an acid-induced lesion of the digestive tract that extends to the submucosa or the mucosal muscle and can generally be located in the lower part of the esophagus or stomach and the upper part of the duodenum (Kuna et al., 2019; Dunlap & Patterson, 2019). It is estimated that it occurs in 5 to 10% of the general population (Kuna et al., 2019) and can trigger serious complications such as bleeding or perforation, with a high risk of mortality due to peritonitis (Sverdén et al., 2019). Peptic ulcer originates from an imbalance between the mechanisms that contribute to mucosal integrity (prostaglandins, nitric oxide, sulfhydryl groups, mucus bicarbonate barrier, and decreased gastric motility) and aggressive factors (Kuna et al., 2019; Dunlap & Patterson, 2019). Risk factors for this disease include *H. pylori* infection, prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs) and other drugs (corticosteroids, potassium chloride, chemotherapeutic agents) as they modify some protective factors of the gastric mucosa, tobacco and alcohol use, stress after an injury or intense physical illness, radiation therapy, viruses, and metabolic disorders. (Dunlap & Patterson, 2019; Kuna et al., 2019). Diagnostic and treatment protocols have not changed in the last 20 years (Brătucu et al., 2021). Proton pump inhibitor drugs (Perry et al., 2020) are the most promising for the treatment of gastric ulcers. However, its prolonged use triggers adverse effects such as gastric hypochlorhydria and hypergastrinemia, alterations in the absorption of calcium, iron, magnesium, and vitamin B_{12}, and/or gastric or pancreatic cancer (Kavitt et al., 2019; Peng et al., 2018), so it is necessary to seek other therapeutic alternatives. Medicinal plants are considered the main reservoir of potential drugs (Kuna et al., 2019) and it has been shown that studies based on ethnobotanical information have produced many useful drugs. However, research validating the empirical use of medicinal plants is still scarce (Jiménez-Suárez et al., 2016). The *Hamelia patens* Jacq. plant (Rubiaceae), commonly known by the names of trumpet, coralillo, or coral grass, which is traditionally used in the state of Chiapas to treat peptic ulcer, there is no scientific research available in this regard, so the objective of this study was to determine the gastroprotective effect of *Hamelia patens* using the model of ethanol-induced gastric lesions in Wistar rats.

METHODOLOGY

Animals

Male Wistar rats weighing between 180-220 g purchased from Universidad Autónoma Metropolitana, Xochimilco campus, Mexico City, were used. The care and management of the animals were carried out by official Mexican guidelines (Mexican Official Standard [NOM-062-ZOO], 1999). The study

was approved by the Internal Committee for the Care and Use of Laboratory Animals (CICUAL) of the Escuela Superior de Medicina del Instituto Politécnico Nacional, with registration number: ESM.CICUAL 14-03-01-2018. The animals were placed in individual cages provided with a metal mesh floor, 24 hours before carrying out the evaluations they were deprived of food, but with free access to water (Sánchez-Mendoza et al., 2022). All experiments were carried out with 7 animals per group.

Vegetal Material

The *Hamelia patens* Jacq. plant (Rubiaceae) was collected in April 2021, in the municipality of Copainalá, Chiapas, Mexico. The plant was identified by biologist Manuel de Jesús Gutiérrez Morales, from the Department of Flora of the CHIP Herbarium, and assigned the registration number: 27762.

Extraction and fractionation

The leaves of *Hamelia patens* were dried at room temperature in the shade and then ground with the help of a mechanical mill. Five kilos of dried and ground leaves were extracted via maceration, for which the leaves were first put in contact with hexane for three days. After this time, the solvent was filtered and concentrated with the help of a rotary evaporator, this operation was repeated twice more to obtain the hexane extract. Subsequently, the plant residue was extracted with dichloromethane and methanol, respectively, following the methodology described above to obtain the dichloromethane and methanol extract (López-Lorenzo et al., 2022). Extracts were evaluated in the ethanol-induced gastric lesion model in Wistar rats. The most active extract, dichloromethane, was separated by silica gel column chromatography with large changes in polarity, giving four fractions. As elution system, F1 = hexane/ethyl acetate 9:1, F2 = hexane/ethyl acetate 8:2, F3 = hexane/ethyl acetate 5:5 and F4 = ethyl acetate 100 % was used.

Ethanol-induced gastric lesions

Extracts (10, 30, and 100 mg/kg), fractions (100 mg/kg), carbenoxolone (3-100 mg/kg), and vehicle 80 Tween 0.05% were administered to Wistar rats orally (0.5 mL/100 g) to the different groups of animals. Thirty minutes later, 1 mL ethanol was administered orally (independent of weight) to all animals to cause gastric lesions. After 2 hours, the animals were sacrificed in a CO_2 chamber. The stomachs were dissected immediately and, subsequently, filled with formaldehyde (2%), five minutes later, they were opened by the greater curvature to measure the area of the gastric lesions with the

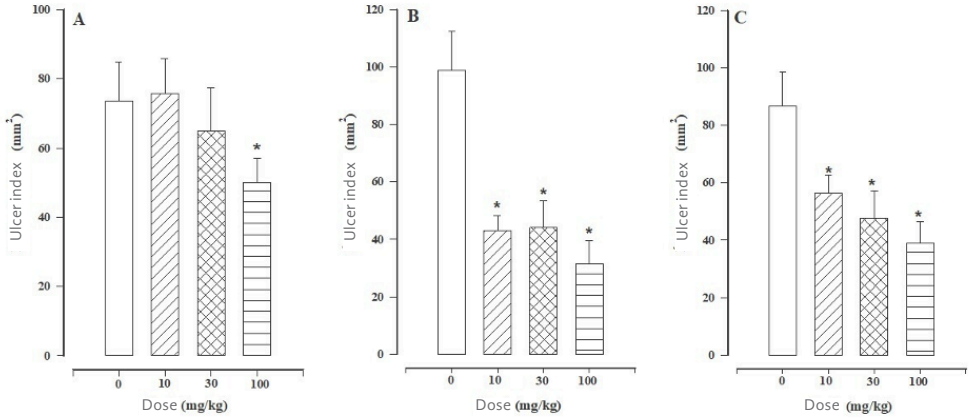
help of a stereoscopic microscope equipped with an ocular micrometer. The ulcer index was calculated as the sum of all lesions in mm2 of each stomach (Sánchez-Mendoza et al., 2022).

Statistical Analysis

Data are expressed as mean ± SEM (Standard error of the mean; n = 7). Differences between treatment groups were analyzed using the Kruskal-Wallis test followed by the Dunn test. The Mann-Whitney U test was used to compare the two groups. A significant difference was considered with a p-value< 0.05.

RESULTS

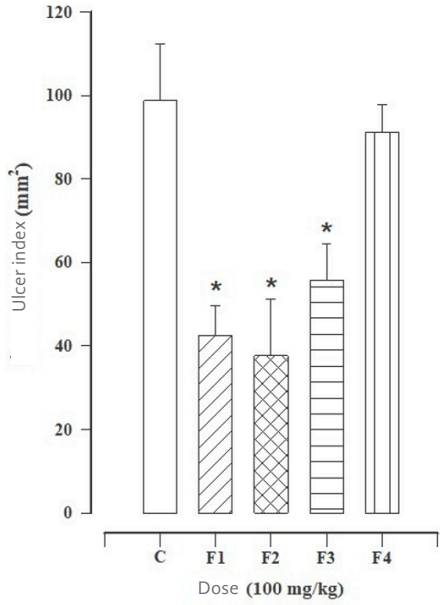
Extracts of hexane, dichloromethane, and methanol from *Hamelia patens* induced a gastroprotective effect against ethanol-induced lesions at the dose of 100 mg/kg (Figure 1 [A]). However, the hexane extract was inactive at lower doses (10 and 30 mg/kg), since there is no significant difference when compared to the vehicle control group. In contrast, dichloromethane and methanol extracts were active at those doses (10 and 30 mg/kg). It should be noted that the effect of these two active extracts is not dose-dependent (Figure 1 [B and C]). In the biodirected study, it was decided to fractionate the dichloromethane extract, since a significant difference between the two was found when comparing it with the methanolic extract at the dose of 10 mg/kg.



Note: Each bar represents the E.E.M.± average. (n = 7) *p < 0.05 compared to their respective control. Kruskal-Wallis test followed by Dunn's multiple comparison test.

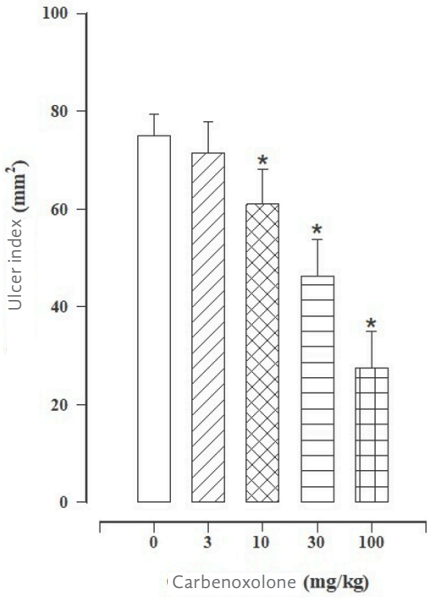
Figure 1. (A, B, and C). Gastroprotective effect of hexane extract (A), dichloromethane (B), and methanol (C) on ethanol-induced gastric lesions in Wistar rats

In the evaluation of the fractions obtained from the dichloromethane extract (Figure 2, 100 mg/kg) it was found that three of them, F1, F2, and F3, were active; and only F4 was inactive. When making a thin layer chromatography of the active fractions, some similar Rf (0.6) were observed between them. Regarding carbenoxolone, it was protected from gastric damage from the 10 mg/kg dose and had a dose-dependent effect (Figure 3). When comparing the ulcer index of the reference drug with that obtained by fractions F1 and F2, no statistical difference was found, indicating that they exert the same protection at the dose of 100 mg/kg.



Note. C=control, F1=hexane/ethyl acetate 9:1, F2=hexane/ethyl acetate 8:2, F3= hexane/ethyl acetate 5:5 and F4= 100% ethyl acetate. Each bar represents the average E.E.M.± (n = 7) *p < 0.05 compared to their respective control. Kruskal-Wallis test followed by Dunn's multiple comparison test.

Figure 2. Gastroprotective effect of dichloromethane extract fractionation on ethanol-induced gastric lesions in Wistar rats



Note. Each bar represents the average E.E.M.± (n = 7) *p < 0.05 compared to their respective control. Kruskal-Wallis test followed by Dunn's multiple comparison test.

Figure 3. Gastroprotective effect of carbenoxolone on ethanol-induced gastric lesions in Wistar rats

DISCUSSION

The results obtained showed that the *Hamelia* patens plant exerts gastroprotective activity, which supports its therapeutic use to treat gastric ulcers in traditional medicine in our country. Additionally, it is worth mentioning that previous studies of *Hamelia* patens have shown antinociceptive and anti-inflammatory activity (Noor et al., 2020). This combination of activities would give *Hamelia* patens an advantage because this would help reduce the doses of NSAIDs in those patients who consume them for a long time and thus reduce the risk of peptic ulcer caused by this type of drug (Dunlap & Patterson, 2019). On the other hand, throughout the biodirected study, it was evident that the plant contains more than one active compound with different physicochemical properties since the activity was found in the three extracts evaluated (hexane, dichloromethane, and methanol). However, the hexane extract only exerted activity at the dose of 100 mg/kg, which can be for two reasons: that said extract has very little of the active constituent (s), or that said compounds are poorly active. In contrast, the gastroprotective effect achieved with the 10 mg/kg dose of the dichloromethane extract differs significantly from the methanolic extract at the same dose, indicating that, in the dichloromethane extract, the compound(s) responsible for said activity is more potent or in greater quantity. Additionally,

from the fractionation of the dichloromethane extract three fractions were active and their thin layer chromatographies showed compounds with similar Rf, which suggests that fractions 1, 2, and 3 probably contain the same compound, in addition to others. Phytochemical studies of the plant *Hamelia* patens have reported that it contains a flavanone glycoside, rosmarinic acid, and various alkaloids such as pteropodine, isopteropodine, rumberine, palmirina, maruquina, alkaloid A, tetrahydroalstonine, aricina, hamelina, uncarina, especiofilina, and ephedrine (Jiménez-Suárez et al., 2016). Considering that gastroprotective activity has been reported for some types of flavonoids and alkaloids, it is pending to continue the study of this plant to identify the active gastroprotective constituents and determine their mechanism of action. Interestingly, the protective effect of active fractions F1 and F2 was similar to the reference drug carbenoxolone at the same dose, suggesting that purification of these fractions is likely to yield more active compounds than carbenoxolone.

CONCLUSION

The *Hamelia patens* plant protects Wistar rats from gastric damage caused by ethanol and contains more than one active compound.

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