International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2016.893.900

Research Article
Gastroprotection of 2,3-epoxyjuanislamin, Isolated from *Calea urticifolia*, Against Ethanol-induced Gastric Lesions in Wistar Rats

[1]Linda Ethel García-Martínez, [1]María Elena Sánchez-Mendoza, [2]Daniel Arrieta-Baez, [3]Leticia Cruz-Antonio, [1]César Miguel Mejía-Barradas, [1]Carmen Ruth Soto-Perulero and [1]Jesús Arrieta

[1]Escuela Superior de Medicina, Instituto Politécnico Nacional, Ciudad de México, México
[2]Centro de Nanociencias y Micro y Nanotecnologías del Instituto Politécnico Nacional, Ciudad de México, México
[3]Facultad de Estudios Superiores Zaragoza, UNAM, Ciudad de México, México

Abstract

Background and Objective: *Calea urticifolia* is used in traditional medicine to treat gastric ulcer but there has not as yet been any scientific report on its therapeutic effects. The aim of this study was tested the gastroprotective activity of *C. urticifolia*. **Materials and Methods:** The gastroprotective activity of *C. urticifolia* was evaluated using bioassay-guided fractionation and the model of ethanol-induced gastric lesions in Wistar rats. The statistical significance between treatments was evaluated by the Kruskal-Wallis test, followed by Dunn's multiple comparison test. **Results:** The results show that *C. urticifolia* has gastroprotective activity. The dichloromethane extract was the most active, yielding 95.7±1.5% gastroprotection with a dose of 30 mg kg^{-1}. Five fractions were obtained from the dichloromethane extract and 2,3-epoxyjuanislamin was isolated from one of them. Rats treated with 2,3-epoxyjuanislamin at doses of 3, 10 and 30 mg kg^{-1} presented 64.63±7.46, 83.03±4.63 and 96.1±1.59% gastroprotection, respectively. The effect of 2,3-epoxyjuanislamin at 30 mg kg^{-1} was not modified by pretreatment with indomethacin, N^G-nitro-L-arginine methyl ester or N-ethylmaleimide. **Conclusion:** The bioassay-guided study of *C. urticifolia* validates of the traditional use of this plant for gastric ulcers treatment. The 2,3-epoxyjuanislamin, isolated from the dichloromethane extract was identified as one of the compounds responsible for this activity and its mechanism of action is apparently not related to prostaglandins, nitric oxide or sulfhydryl groups.

Key words: *Calea urticifolia*, 2,3-epoxyjuanislamin, calealactones, sesquiterpene lactone, germacrane, gastroprotection, gastric ulcer, medicinal plants

Received: September 06, 2016 Accepted: October 04, 2016 Published: October 15, 2016

Citation: Linda Ethel García-Martínez, María Elena Sánchez-Mendoza, Daniel Arrieta-Baez, Leticia Cruz-Antonio, César Miguel Mejía-Barradas, Carmen Ruth Soto-Perulero and Jesús Arrieta, 2016. Gastroprotection of 2,3-epoxyjuanislamin, isolated from *Calea urticifolia*, against ethanol-induced gastric lesions in wistar rats. Int. J. Pharmacol., 12: 893-900.

Corresponding Author: Jesús Arrieta, Escuela Superior de Medicina, Instituto Politécnico Nacional, Plan de San Luis y Díaz Mirón, Colonia Santo Tomás, Delegación Miguel Hidalgo, 11340 Ciudad de México, México Tel: +55-5729-6300/62827 Fax: +55-5622-5329

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Peptic ulcers have a worldwide presence and have been a serious health problem for many years. About 10% of the population suffers from this condition at some time in their life[1]. Included in this disease are gastric ulcers and duodenal ulcers, with the former producing a higher mortality.

The pathophysiology of peptic ulcers involves an imbalance between offensive factors, including acid and pepsin and defensive ones, such as prostaglandins, nitric oxide, mucus and bicarbonate[2]. Other factors that can lead to this disorder are ethanol, stress, smoking, nutritional deficiencies and frequent ingestion of nonsteroidal anti-inflammatory drugs (NSAIDs).

The importance of this disease has led to the development of many pharmacological treatments[3]. However, all of the drugs currently on the market are associated with undesirable side effects. For instance, some studies have shown that prolonged use of proton pump inhibitors such as omeprazole may lead to a decrease in the absorption[4] of vitamin B_{12}, myocardial infarction[5] and the risk of chronic kidney disease[6]. Hence, there is a need for safer and more effective anti-ulcer agents with fewer and less pronounced side effects. One alternative therapy for treating ulcers is the use of medicinal plants. Indeed, some of the metabolites derived from plants and used in traditional medicine have provided an important basis for the discovery and development of modern drug therapy[7].

In this study, *Calea urticifolia* (Miller) is used as a folk medicine in the treatment of gastric ulcers in El Salvador and Mexico[8,9]. However, there has as yet been no scientific report concerning this therapeutic practice. Therefore, we tested the gastroprotective activity of *C. urticifolia*, beginning with bioassay-guided fractionation in order to identify the active extracts. The resulting extracts and fractions were tested with the model of gastric lesions induced by absolute ethanol in Wistar rats. After finding the most active extract and fractions, one compound with significant gastroprotective activity was identified as 2,3-epoxyjuanislamin. The mechanism of action of this compound was explored by evaluating the role of prostaglandins, endogenous NO and sulfhydryl groups.

MATERIALS AND METHODS

Animals: All the experiments were performed with male Wistar rats weighing 180-220 g, obtained from the animal house of the Universidad Nacional Autónoma de México, FES-Zaragoza, Mexico City, Mexico. Procedures involving animals and their care were conducted in conformity with the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999) and in compliance with international rules on care and use of laboratory animals. Unless otherwise specified, the rats were placed in single cages with wire-net floors and deprived of food 24 h before experimentation. Animals were allowed free access to tap water throughout the experimental procedures. All experiments were carried out with 7 animals per group.

Drugs: The drugs, extracts, fractions and 2,3-epoxyjuanislamin were prepared immediately before use. Carbenoxolone (the reference drug), N^G-nitro-L-arginine methyl ester (L-NAME), indomethacin and N-ethylmaleimide (NEM) were purchased (Sigma-Aldrich Co., St. Louis, MO, USA).

Plant material: During July of 2014, the leaves of *C. urticifolia* were collected in Nandayalud, a community in the municipality of Suchiapa, the State of Chiapas, Mexico. The plant was identified and registered by Manuel de Jesús Gutiérrez Morales from the Flora Department of the Chip Herbarium, which is part of the Botanical Garden of the Secretary of Environmental Protection, Housing and Natural History of the State of Chiapas, Mexico. A specimen of the original collection can be found with the voucher number 39871.

Extraction and preliminary fraction: About 2.7 kg of dried and ground leaves were extracted by reflux. Extractions were performed two times during 3 h, first with hexane (11 L), then dichloromethane (11 L) and finally methanol (11 L). Evaporation of the solvents in vacuum yielded 102.2, 150 and 250 g of syrupy residues, respectively. The dichloromethane extract obtained from the leaves of *C. urticifolia* showed the greatest gastroprotective effect (Table 1). Thus, this extract (140 g) was subjected to fractionation by a silica gel column using a step gradient of hexane (5 L, F1) hexane/EtOAc (9:1, 5 L, F2), hexane/EtOAc (7:3, 5 L, F3), hexane/EtOAc (1:1, 5L, F4) and EtOAc (5L, F5).

The results of evaluation of these fractions indicated that two are active (F3 and F4), without any significant difference between their gastroprotective activity. Since the yield of F3 (15 g) was lower than that of F4, we decided to work with the latter fraction. Hence, 35 g of F4 were chromatographed on a silica gel column by using a step gradient of hexane, hexane/EtOAc and EtOAc. From this procedure, we obtaining three fractions (F1′, F2′ and F3′), of which the fraction F2′ was the most active (92.1±4.3% gastroprotection at a dose of 30 mg kg^{-1}). A portion (14 g) of this fraction was

Table 1: Gastroprotective effect of the extracts of *Calea urticifolia*

Treatment	Dose (mg kg^{-1})	n	UI (mm^2)	Gastroprotection (%)
Control	--	7	93.3±7.0	--
Hexane extract	30	7	77.2±9.5	17.2±10.2
	100	7	30.6±7.0*	67.1±7.5
Dichloromethane extract	1	7	53.0±5.2*	42.8±5.5
	3	7	47.2±5.3*	49.3±5.7
	10	7	39.3±5.1*	57.8±5.0
	30	7	4.0±1.4*	95.7±1.5
	100	7	1.5±0.5*	98.3±0.5
Methanol extract	30	7	24.1±6.7*	74.1±7.2
	100	7	5.8±2.2*	93.7±2.4

*p<0.05 vs control group, UI: Ulcer index

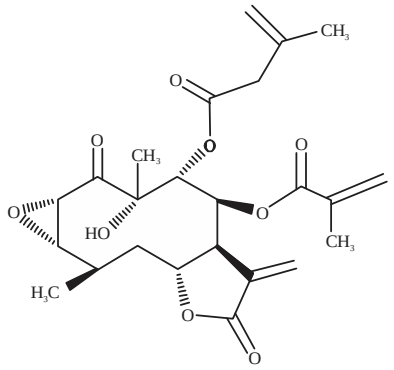

Fig. 1: 2,3-epoxyjuanislamin

subjected again to chromatography to obtain white crystals, which had a melting point of 146-148°C. This substance was identified as 2,3-epoxyjuanislamin (Fig. 1) by comparing the ^{1}H and ^{13}C NMR spectra to those reported in the literature[8].

Ethanol-induced gastric mucosal injury: The different treatments with the extracts, fractions, 2,3-epoxyjuanislamin (suspended in 0.5% tween 80) or carbenoxolone (dissolved in water) were administered orally (0.5 mL/100 g) 30 min before administration of 1 mL of ethanol by same route. Two hours later the animals were sacrificed in a CO_2 chamber and the stomachs were immediately removed, inflated with formalin and placed in 2% formalin for 5 min before being opened along the greater curvature to quantify gastric lesions. The ulcer index was calculated as the sum of all lesions (area in mm^2) in the stomach of each animal and then gastroprotection (%) was calculated according to the method proposed by Sanchez-Mendoza *et al.*[10]. The control group included in these assessments was treated with the vehicle followed by ethanol.

Ethanol-induced gastric mucosal lesions in indomethacin pretreated rats: To assess whether endogenous prostaglandins are involved in the gastroprotective effect of 2,3-epoxyjuanislamin, a control group received a subcutaneous injection of $NaHCO_3$ (5 mM) dissolved in saline and 3 more groups were treated with indomethacin (10 mg kg^{-1} dissolved in $NaHCO_3$ 5 mM) by the same route. After waiting 75 min, the control group was treated orally with tween 80 (0.05%) and the other groups received one of three oral treatments: Tween 80 (0.05%), 30 mg kg^{-1} of 2,3-epoxyjuanislamin, or 100 mg kg^{-1} of carbenoxolone. Thirty minutes later, all the animals received 1 mL of absolute ethanol and 2 h afterwards the rats were euthanized in a CO_2 chamber. Stomachs were removed and the ulcer index was measured[10] as aforementioned.

Ethanol-induced gastric mucosal lesions in L-NAME pretreated rats: To investigate the involvement of endogenous NO, L-NAME was intraperitoneally administered (70 mg kg^{-1} dissolved in saline) to 3 groups of animals 30 min before oral administration of the vehicle, 2,3-epoxyjuanislamin (30 mg kg^{-1}) or carbenoxolone (100 mg kg^{-1}). After waiting 30 min, 1 mL ethanol was administered to all groups and 2 h later the animals were sacrificed to measure the ulcer index. A control group not treated with L-NAME was included in this evaluation[10].

Ethanol-induced gastric mucosal lesions in NEM pretreated rats: To determine the involvement of endogenous sulfhydryl groups in the gastroprotective activity, NEM (10 mg kg^{-1} dissolved in saline) was administered subcutaneously to 3 groups of animals 30 min before oral administration of the vehicle, 2,3-epoxyjuanislamin (30 mg kg^{-1}) or carbenoxolone (100 mg kg^{-1}). After waiting 30 min, all animals were given 1 mL of absolute ethanol and 2 h later they were sacrificed to determine the ulcer index. A control group not treated with NEM was included in this evaluation[10].

Statistical analysis: Data are presented as the Mean±SEM of 7 rats per group. The statistical significance between

treatments was evaluated by the Kruskal-Wallis test, followed by Dunn's multiple comparison test with $p \leq 0.05$ considered as significant.

RESULTS

Bioassay-guided fractionation and isolation of 2,3-epoxyjuanislamin: Different extracts of *C. urticifolia* were administered orally at distinct doses to establish their gastroprotective activity on gastric lesions induced by ethanol. Table 1 shows that all three extracts showed gastroprotective activity and the dichloromethane extract was the most active, reaching 95.7±1.5% gastroprotection with a dose of 30 mg kg^{-1}. A similar value was obtained with the methanol extract, obtaining 93.7±2.4% gastroprotection. However, this effect required a dose of 100 mg kg^{-1}. Finally, the hexane extract was the least active, with only 67.1±7.5% gastroprotection at a dose of 100 mg kg^{-1}.

In the evaluation of the fractions obtained from the dichloromethane extract, it was found that only the F3 and F4 fractions inhibited gastric lesions (compared to the control group). The gastroprotection obtained for F3 and F4 at a dose of 30 mg kg^{-1} was 92.4±3.4 and 88.1±4.3%, respectively (Table 2), with an insignificant difference between these two values. Since F4 was obtained in a higher yield, silica gel column chromatography was employed to separate this fraction, finding that one of the isolated compounds was responsible for the gastroprotective activity of this fraction. This pure compound (1.2 g) was identified as 2,3-epoxyjuanislamin (Fig. 1), which presented a dose-dependent effect (Fig. 2). It reached its maximum effect (96.1±1.59%) at a dose of 30 mg kg^{-1}. On the other hand, the reference drug required a dose of 100 mg kg^{-1} to exhibit its maximum effect (68.5±8.02%).

Ethanol-induced gastric mucosal lesions with indomethacin, L-NAME and NEM pretreatments: For the rats treated with 10 mg kg^{-1} indomethacin, 70 mg kg^{-1} of L-NAME or 10 mg kg^{-1} of NEM and then administered ethanol, the ulcer index values (114±7.75, 112±6.79 and 117±9.35 mm^2, respectively) were not significantly different from that of the vehicle control group (106.05±9.42 mm^2). This is in accordance with previous reports that these inhibitors do not produce gastric damage at the doses herein employed[11].

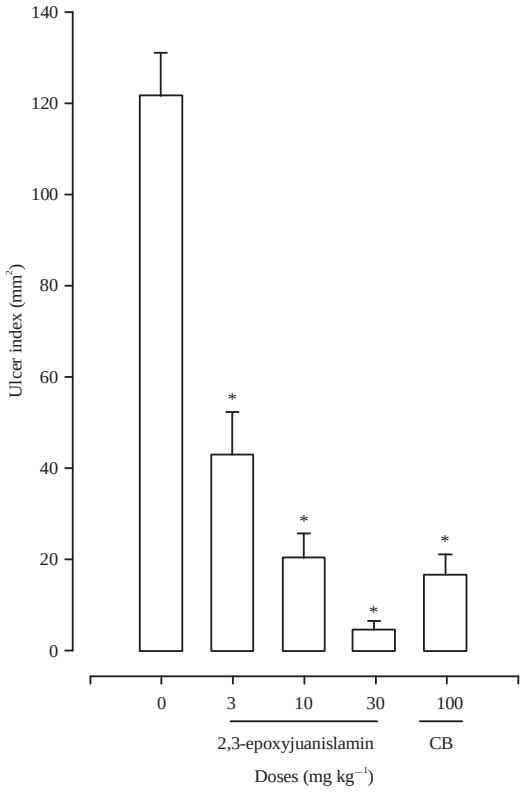

Fig. 2: Gastroprotective effect of 2,3-epoxyjuanislamin (3-30 mg kg^{-1}) or carbenoxolone (CB: 100 mg kg^{-1}). Bars represent the Mean±SEM (n = 8). *p<0.05 vs the respective control, Dunn's multiple comparison test after the Kruskal-Wallis test

Table 2: Gastroprotective effect of the fractions of the dichloromethane extract

Treatment	Dose (mg kg^{-1})	n	UI (mm^2)	Gastroprotection (%)
Control	--	7	83.0±7.4	--
F1	30	7	85.7±6.4	--
F2	30	7	81.0±9.6	2.3±11.5
F3	30	7	6.2±2.8*	92.4±3.40
F4	30	7	8.1±2.8*	88.1±4.30
F5	30	7	70.1±8.6	15.4±10.4

*p<0.05 vs control group, UI: Ulcer index

The ulcer index obtained in rats pretreated with indomethacin, L-NAME or NEM and then later treated with 2,3-epoxyjuanislamin were 41.16±8.99, 8.16±2.21 and 12.91±6.71 mm^2, respectively. These values were significantly different ($p \leq 0.05$) when compared with the vehicle control (106.05±9.42 mm^2), meaning that neither of the pretreatments modified the gastroprotective effect of 2,3-epoxyjuanislamin at 30 mg kg^{-1} (Fig. 3a-c). On the other hand, pretreatment with indomethacin, L-NAME or NEM followed by the carbenoxolone treatment resulted in an ulcer index of 96.41±3.99, 94.81±7.65 and 96.62±8.53 mm^2,

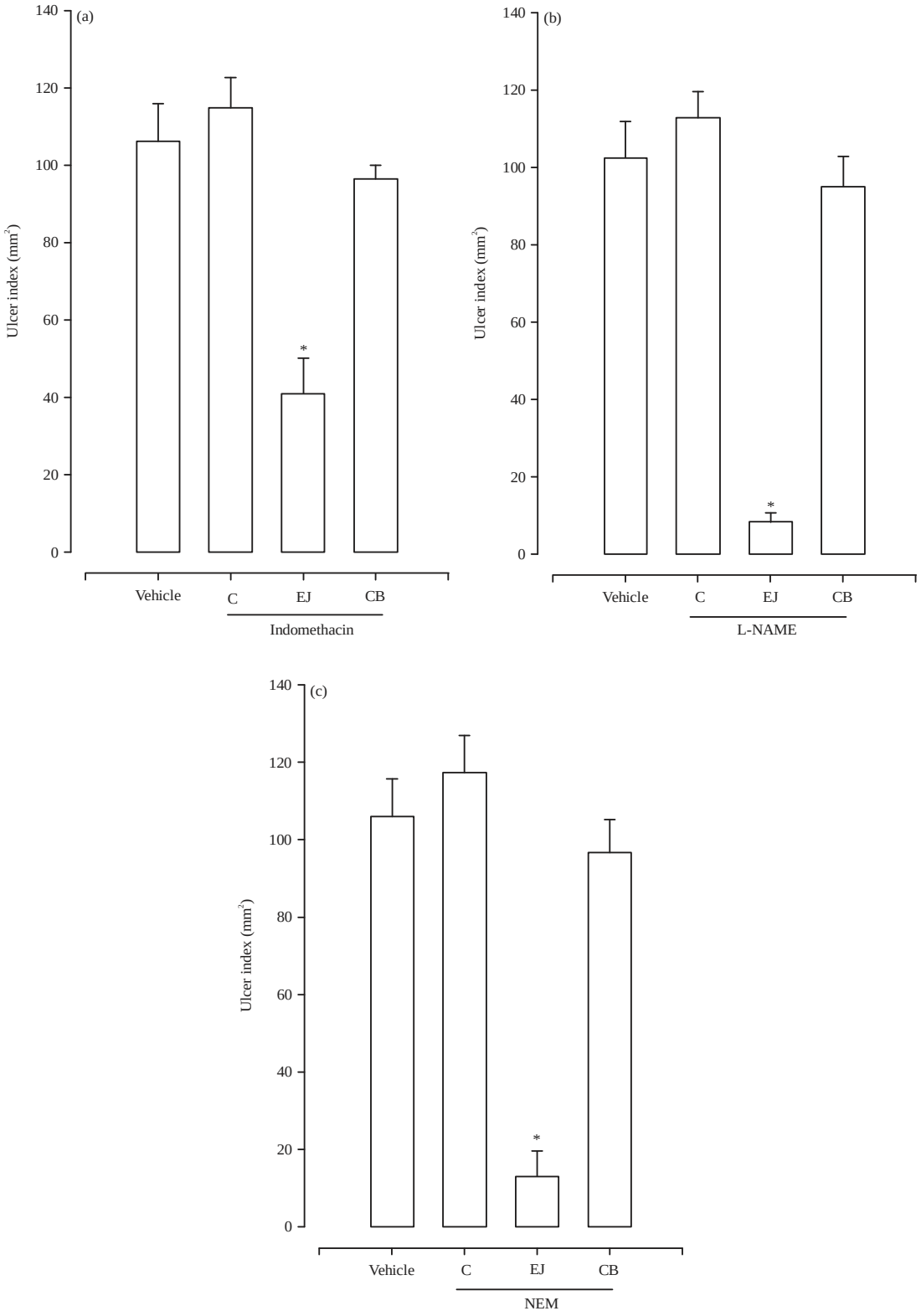

Fig. 3(a-c): Gastroprotective effect of 2,3-epoxyjuanislamin (EJ, 30 mg kg^{-1}) or carbenoxolone (CB, 100 mg kg^{-1}) in rats pretreated with (a) Indomethacin (10 mg kg^{-1}), (b) L-NAME (70 mg kg^{-1}) and (c) NEM (10 mg kg^{-1}). Bars represent the Mean±SEM (n = 8). *p<0.05 vs the respective control, Dunn's multiple comparison test after the Kruskal-Wallis test

respectively. These values indicate that the pretreatments did not differ significantly from the control group, meaning that prostaglandins, nitric oxide and sulfhydryl groups are probably all involved in the mechanism of action of this compound. These results agree with reports in the literature.

DISCUSSION

In the present study, the gastroprotective activity of *C. urticifolia* was evaluated through a bioassay-guided study, using the model of ethanol-induced gastric lesions in rats. This model is widely used in the search for new compounds to treat gastric ulcer, because by modifying the mucus layer in the stomach, ethanol causes necrotic lesions[10].

The bioassay-guided study of *C. urticifolia* showed that the hexane, dichloromethane and methanol extracts all had gastroprotective activity (Table 1), indicating that this plant has more than one active compound. The dichloromethane extract was the most active against gastric lesions, obtaining 95.7±1.5% gastroprotection with a 30 mg kg^{-1} dose.

By fractionation of the dichloromethane extract, it was found that only F3 and F4 were active (Table 2), indicating that at least two compounds are responsible for the activity of this extract. Since the gastroprotection was not significantly different between these two fractions, further study was conducted with F4 because it was obtained with a higher yield. From F4, 2,3-epoxyjuanislamin was isolated and identified as being responsible for the gastroprotective activity of this fraction. This compound is a sesquiterpene lactone and has the same biological activities previously observed with other germacranolides isolated from *C. urticifolia*, such as inhibition of melanin biosynthesis in mouse B16 melanoma cells, apoptosis induction in HL60 cells and suppressive activity against adipocyte differentiation[9]. Additionally, the present study shows for the first time that 2,3-epoxyjuanislamin produces gastroprotective activity in the model of gastric lesions induced by ethanol (Fig. 2).

The effect of 2,3-epoxyjuanislamin was found to be dose dependent, presenting considerable activity with a low dose (3 mg kg^{-1}) and reaching its maximum effect (96.10±1.59% gastroprotection) at the dose of 30 mg kg^{-1}. It has been reported that tagitinin C, another sesquiterpene lactone with a skeleton of germacrane, also has gastroprotective activity in the same model. Indeed, tagitinin C is slightly more potent than 2,3-epoxyjuanislamin, reaching 100% gastroprotection[12] at a dose of 10 mg kg^{-1}. Hence, the substituents of tagitinin C are advantageous for gastroprotective activity in comparison to 2,3-epoxyjuanislamin.

Some studies indicate that *C. urticifolia* contains several types of sesquiterpene lactones with a germacrane skeleton[8,13], which could explain the biological activity of the three extracts presently isolated. It is therefore possible that this same type of compound is responsible for the activity of F3.

The compounds that help to protect the gastric mucosa against ethanol-induced damage act primarily by stimulating defense mechanisms or inhibiting aggressive factors. Therefore, the present study explored three possible mechanisms of action of 2,3-epoxyjuanislamin in relation to protective factors of the gastric mucosa: Prostaglandins, Nitric Oxide (NO) and non-protein sulfhydryl groups.

It is well known that endogenous prostaglandins (PGs) are significantly involved in maintaining the integrity of the gastric mucosa against various harmful agents[14]. Anti-inflammatory drugs (NSAIDs), such as indomethacin, provoke gastric damage because they non selectively inhibit enzymes (COX) responsible for the biosynthesis of PGs[15]. Pretreatment with indomethacin at a concentration that inhibits COX but does not produce gastric damage, did not modify the gastroprotective effect of 2,3-epoxyjuanislamin, suggesting that PGs are not involved in the mechanism of action of 2,3-epoxyjuanislamin (Fig. 3a).

The NO is another compound that plays an important role in the integrity of the gastric mucosa in many tissues, including the gastrointestinal tract. Previous studies report that ulcerogenic responses may be mediated by NO, a molecule produced mainly by iNOS[16]. In the present study, the administration of L-NAME (an unspecific inhibitor of NOS) did not reverse the protective effect of 2,3-epoxyjuanislamin, indicating that NO is not involved in the mechanism of action of this compound (Fig. 3b).

Also, helping to maintain the integrity of the gastric mucosa are endogenous sulfhydryl groups such as glutathione. It is known that these compounds participate in maintaining the mucus layer by binding its subunits through disulfide bridges. Additionally, sulfhydryl groups may act as antioxidants by eliminating free radicals and/or regulate the synthesis and avoid the degradation of proteins, in part by maintaining their structure[10]. In this study, NEM (an SH blocker) was administered at a dose capable of blocking of

sulfhydryl groups but not of producing gastric damage. It was herein found that NEM pretreatment did not reduce the gastroprotective effect of 2,3-epoxyjuanislamin, suggesting that endogenous sulfhydryl groups are not involved in the mechanism of action of this compound (Fig. 3c).

Studies on the antioxidant activity of sesquiterpene lactones of *C. urticifolia* indicate that these compounds interact with sulfhydryl groups of the cysteine residues of the protein Keap1. This reaction causes conformational changes that lead to the release of cytoplasmic Nrf2 and its subsequent translocation to the nucleus, where it joins ARE sites (Nrf2/ARE) in the promoter region of genes encoding for antioxidant enzymes[17]. This suggests the possibility that 2,3-epoxyjuanislamin interacts with sulfhydryl groups of the cysteine residues of ATPase H^+/K^+, in the same way that metabolites of omeprazole act to produce their antisecretory activity. However, further studies are necessary to corroborate this hypothesis.

CONCLUSION

The present study shows the first scientific evidence supporting the traditional use of *Calea urticifolia* to treat gastric ulcers. The 2,3-epoxyjuanislamin was identified as one of the compounds responsible for this activity and its mechanism of action is apparently not related to prostaglandins, nitric oxide or sulfhydryl groups. More studies should be carried out to determine the possible mechanism of action of 2,3-epoxyjuanislamin and also isolate other active compounds from this plant.

ACKNOWLEDGMENT

We are grateful to Manuel de Jesús Gutiérrez Morales for collecting the plant material. This study was supported by two grants from the School of Medicine of the National Polytechnic Institute, Mexico (SIP 20161093 and SIP 20160919).

REFERENCES

1. Rojas-Martínez, R., J. Arrieta, L. Cruz-Antonio, D. Arrieta-Baez, A.M. Velázquez-Méndez and M.E. Sánchez-Mendoza, 2013. Dillapiole, isolated from *Peperomia pellucida*, shows gastroprotector activity against ethanol-induced gastric lesions in Wistar rats. Molecules, 18: 11327-11337.
2. Song, J.L., Y. Zhou, X. Feng and X. Zhao, 2015. White tea (*Camellia sinenesis* (L.)) ethanol extracts attenuate reserpine-induced gastric ulcers in mice. Food Sci. Biotechnol., 24: 1159-1165.
3. Miranda, M.A., M. Lemos, K.A. Cowart, D. Rodenburg and J.D. McChesney *et al*., 2015. Gastroprotective activity of the hydroethanolic extract and isolated compounds from the leaves of *Solanum cernuum* Vell. J. Ethnopharmacol., 172: 421-429.
4. Lam, J.R., J.L. Schneider, W. Zhao and D.A. Corley, 2013. Proton pump inhibitor and histamine 2 receptor antagonist use and vitamin B_{12} deficiency. J. Am. Med. Assoc., 310: 2435-2442.
5. Shah, N.H., P. LePendu, A. Bauer-Mehren, Y.T. Ghebremariam and S.V. Iyer *et al*., 2015. Proton pump inhibitor usage and the risk of myocardial infarction in the general population. PLoS ONE, Vol. 10. 10.1371/journal.pone.0124653
6. Lazarus, B., Y. Chen, F.P. Wilson, Y. Sang, A.R. Chang, J. Coresh and M.E. Grams, 2016. Proton pump inhibitor use and the risk of chronic kidney disease. JAMA Internal Med., 176: 238-246.
7. Babu, T.H., K. Manjulatha, G.S. Kumar, A. Hymavathi and A.K. Tiwari *et al*., 2010. Gastroprotective flavonoid constituents from *Oroxylum indicum* Vent. Bioorg. Med. Chem. Lett., 20: 117-120.
8. Yamada, M., N. Matsuura, H. Suzuki, C. Kurosaka and N. Hasegawa *et al*., 2004. Germacranolides from *Calea urticifolia*. Phytochemistry, 65: 3107-3111.
9. Torres-Rodríguez, M.L., E. García-Chávez, M. Berhow and E.G. de Mejia, 2016. Anti-inflammatory and anti-oxidant effect of *Calea urticifolia* lyophilized aqueous extract on lipopolysaccharide-stimulated RAW 264.7 macrophages. J. Ethnopharmacol., 188: 266-274.
10. Sánchez-Mendoza, M.E., L. Cruz-Antonio, D. Arrieta-Baez, I.M. Olivares-Corichi, R. Rojas-Martínez, D. Martínez-Cabrera and J. Arrieta, 2015. Gastroprotective activity of methyleugenol from *Peperomia hispidula* on ethanol-induced gastric lesions in rats. Int. J. Pharmacol., 11: 697-704.
11. Reyes-Trejo, B., M.E. Sánchez-Mendoza, A.A. Becerra-García, E. Cedillo-Portugal, C. Castillo-Henkel and J. Arrieta, 2008. Bioassay-guided isolation of an anti-ulcer diterpenoid from *Croton reflexifolius*: Role of nitric oxide, prostaglandins and sulfhydryls. J. Pharm. Pharmacol., 60: 931-936.
12. Sánchez-Mendoza, M.E., A. Reyes-Ramírez, L. Cruz-Antonio, L. Martínez-Jiménez, J. Rodríguez-Silverio and J. Arrieta, 2011. Bioassay-guided isolation of an anti-ulcer compound, tagitinin C, from *Tithonia diversifolia*: Role of nitric oxide, prostaglandins and sulfhydryls. Molecules, 16: 665-674.

13. Del Castillo, J.B., M.T.M. Ferrero, F.R. Luis, P.V. Bueno, N.G. Leonor and S.C. Arevalo, 1981. Salvadorian compositae. II. Juanislamin and 2,3-epoxy-juanislamin, two new sesquiterpenic lactones from *Calea urticifolia*. J. Nat. Prod., 44: 348-350.
14. Khattab, M.M., M.Z. Gad and D. Abdallah, 2001. Protective role of nitric oxide in indomethacin-induced gastric ulceration by a mechanism independent of gastric acid secretion. Pharmacol. Res., 43: 463-467.
15. Gupta, M. and G.M. Eisen, 2009. NSAIDs and the gastrointestinal tract. Curr. Gastroenterol. Rep., 11: 345-353.
16. Nishida, K., Y. Ohta and I. Ishiguro, 1998. Contribution of NO synthases to neutrophil infiltration in the gastric mucosal lesions in rats with water immersion restraint stress. FEBS Lett., 425: 243-248.
17. Umemura, K., T. Itoh, N. Hamada, Y. Fujita and Y. Akao *et al*., 2008. Preconditioning by sesquiterpene lactone enhances H_2O_2-induced Nrf2/ARE activation. Biochem. Biophys. Res. Commun., 368: 948-95.