

Gastroprotective activity of caryophyllene oxide: the role of nitric oxide, prostaglandins and sulfhydryls

María Elena Sánchez-Mendoza¹, Leticia Cruz-Antonio², María Guadalupe Cupido-Sánchez¹, Guillermo García-Castillo¹, Jesús Arrieta^{1*}

¹Escuela Superior de Medicina. Instituto Politécnico Nacional. Plan de San Luis y Díaz Mirón. Colonia Santo Tomás, Delegación Miguel Hidalgo, 11340, México, D. F. Mexico. ²Facultad de Estudios Superiores Zaragoza, UNAM, Batalla del 5 de Mayo Esquina Fuerte de Loreto, Ejército de Oriente, México D.F., 09230, Mexico.

ARTICLE INFO

Article history:

Received on: 14/08/2014

Revised on: 27/08/2014

Accepted on: 11/09/2014

Available online: 27/09/2014

Key words:

caryophyllene oxide;
terpenoid; gastroprotection;
gastric ulcer.

ABSTRACT

The present study was carried out to evaluate the gastroprotective effect of caryophyllene oxide, and investigate the gastroprotective mechanism. For this purpose, Wistar rats received vehicle, caryophyllene oxide (10-100 mg/kg) or carbenoxolone (1-100 mg/kg, used as the reference drug). Thirty minutes later absolute ethanol was given orally, and 2 h later the stomach was dissected and the damaged area measured. In other experiments, the rats received L-NAME (70 mg/kg, i. p.), indomethacin (10 mg/kg, s. c.), and N-ethylmaleimide (10 mg/kg, s. c.), before the administration of the caryophyllene oxide (100 mg/kg) or carbenoxolone (100 mg/kg). Two control groups were included in these evaluations. Again, thirty minutes later absolute ethanol was given orally, and 2 hours later the animals were sacrificed to measure the ulcer index. Treatment of rats with caryophyllene oxide and carbenoxolone elicited a dose-dependent gastroprotective effect. The gastroprotection observed with the administration of caryophyllene oxide was attenuated in rats pretreated with the inhibitors. This suggests that the gastroprotective mechanism of action of caryophyllene oxide involves NO, prostaglandins and sulfhydryl groups. In the case of carbenoxolone, the partial participation of NO, prostaglandins and sulfhydryls was observed.

INTRODUCTION

A peptic ulcer is a tissue defect in the gastric or duodenal wall that results from a necrotizing event and extends through the *muscularis mucosae* into the deeper layers. It is within these layers that the ulcerative process may erode a major blood vessel and elicit important bleeding (Yeomans and Naesdal, 2008). It is estimated that at some time in life, nearly 20% of all people suffer from a peptic ulcer (Vonkeman *et al.*, 2007). This disorder is thought to result from an imbalance between potentially injurious and protective factors of the gastric mucosa. The irritants can be exogenous or endogenous, such as enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis, excessive bile acids, food ingredients, *Helicobacter pylori*, diminished gastric blood flow and gastric motility (Toma *et al.*, 2005). The pathogenesis of gastric ulcer can also be due to stress or the

ingestion of certain substances, including nonsteroidal anti-inflammatory drugs (NSAIDs), alcohol, tobacco and caffeine. Even normal acid secretion can cause ulceration if gastroprotective factors are overwhelmed (Jainu and Devi, 2006). Current treatment options for patients suffering from gastrointestinal ulceration include cytoprotective drugs, antacids, and in particular, histamine-2 receptor antagonists and proton pump inhibitors. However, it has been observed that long-term use of histamine-2 receptor antagonists and proton pump inhibitors induces innumerable adverse effects. Therefore, there is a need to search for more effective and safer anti-gastric ulcer agents with less side effects. Metabolites derived from plants have provided an important basis for the discovery and development of modern therapeutic drugs (Vera-Arzave *et al.*, 2012). Some natural products derived from plants have shown anti-inflammatory, analgesic and gastroprotector activity. In this context, caryophyllene oxide presents pharmacological activity, including cytotoxic (Jun *et al.*, 2011), analgesic and anti-inflammatory activity (Chavan *et al.*, 2010), as well as a synergistic effect of terpenoids against the epimastigote

* Corresponding Author

Jesús Arrieta, E-Mail: jearrval@yahoo.com.mx;

Tel.: +(55) 57 29 63 00 Ext. 62 827; Fax: +(55) 56 22 53 29.

forms of *T. cruzi* (Polanco-Hernández *et al.*, 2013). Therefore, we decided to test the gastroprotective activity of caryophyllene oxide by using an animal model of gastric lesions induced by absolute ethanol. Regarding the mechanism of action of the gastroprotective effect of caryophyllene, the role of endogenous NO, sulfhydryl groups and prostaglandins was evaluated.

MATERIAL AND METHODS

Animals

All experiments were performed with male Wistar rats, weighing 180–220 g, obtained from the animal house of the Universidad Autónoma Metropolitana, Xochimilco campus, Mexico City, Mexico.

Procedures involving animals and their care were conducted in accordance 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 8–10 animals per group.

Drugs and dosage

Caryophyllene oxide, prepared freshly for each use, was suspended in 0.5% Tween 80 and administered by the intragastric route. Control rats received the vehicle (0.5% Tween 80) in the same volume (0.5 mL/100 g) and by the same route. Carbenoxolone (the reference drug), N^G-nitro-L-arginine methyl ester (L-NAME), N-ethylmaleimide (NEM), indomethacin (IND) and (-)-caryophyllene oxide were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Acute gastric ulcer induced by absolute ethanol

A gastric ulcer was induced by orally administering absolute ethanol (1 mL) (Sánchez-Mendoza *et al.*, 2011). Thirty min before the ethanol administration, one of the compounds (the vehicle, 10–100 mg/kg caryophyllene oxide or 1–100 mg/kg carbenoxolone) was administered to each group by the oral route. Then two hours after the ethanol administration, the animals were sacrificed in a CO₂ chamber. The stomach and duodenum were dissected, inflated with formalin (10 mL), and then placed in 2% formalin for 5 min to fix both the inner and outer layers. The duodenum was opened along its anti-mesenteric side and the stomach along the greater curvature. The damaged area (mm²) was measured under a dissection microscope (×10) with an ocular micrometer. The ulcer index was calculated as the sum of all the lesions (area in mm²) in the stomach of each animal. Gastroprotection (%) was calculated according to the following formula:

$$\% \text{ Gastroprotection} = (\text{UIC} - \text{UIT}) \times 100/\text{UIC}$$

where UIC and UIT are the ulcer indexes of the control and test animals, respectively (Sánchez-Mendoza *et al.*, 2011).

Ethanol-induced gastric mucosal lesions in L-NAME pretreated rats

To assess the participation of endogenous NO in the gastroprotective effect of the test and reference compounds, L-NAME (70 mg/kg dissolved in saline solution) was intraperitoneally injected into the animals 30 min before the administration of the vehicle, caryophyllene oxide or carbenoxolone (Sánchez-Mendoza *et al.*, 2011). A control group received no L-NAME pretreatment. Thirty minutes after treatment with one of the test compounds, absolute ethanol was given to each rat in these four groups. The animals were sacrificed 2 h after the administration of ethanol to measure the ulcer index.

Ethanol-induced gastric mucosal lesions in indomethacin pretreated rats

To assess the participation of endogenous prostaglandins in the gastroprotective effect of the test compounds, rats were subcutaneously injected with indomethacin (10 mg/kg dissolved in NaHCO₃ at 5 mM). A control group received an injection of 5 mM NaHCO₃ in saline solution by the same route. After 75 min, subgroups of animals in each of these two groups (with and without indomethacin pretreatment) received one of three oral treatments (saline solution, 100 mg/kg caryophyllene oxide or 100 mg/kg carbenoxolone) (Sánchez-Mendoza *et al.*, 2011). Absolute ethanol was given to each rat 30 min after one of these three treatments and rats were sacrificed 2 h later in a CO₂ chamber. The stomachs were subsequently removed to measure the ulcer index, as aforementioned.

Ethanol-induced gastric mucosal lesions in NEM pretreated rats

To assess the participation of endogenous sulfhydryls in the gastroprotective effect of the test and reference compounds, NEM (dissolved in saline solution) was subcutaneously injected (10 mg/kg) in 3 groups of animals 30 min before the oral administration of the vehicle, caryophyllene oxide or carbenoxolone (Sánchez-Mendoza *et al.*, 2011). A control group received no treatment. Thirty minutes after treatment with one of the three compounds, absolute ethanol was given to each rat and animals were sacrificed 2 h later to measure the intensity of the gastric ulcer.

Statistical analysis

Data are presented as the mean ± SEM of 8 to 10 rats per group. Statistical significance between treatments was evaluated by the Kruskal-Wallis test, followed by Dunn's multiple comparison tests, with p ≤ 0.05 considered as significant.

RESULTS

Gastroprotection of caryophyllene oxide

Compared with the control group, caryophyllene oxide (10–100 mg/kg) reduced the ethanol-induced gastric haemorrhagic lesions in a dose-dependent manner (Figure 1A). Treatment with

caryophyllene oxide elicited a gastroprotective effect of 13, 31, 49, 63 and 77% at doses of 10, 16, 30, 54.7 and 100 mg/kg, respectively. Likewise, treatment with carbenoxolone (the reference drug) also showed a dose-dependent gastroprotective effect of 25, 36, 51, 71 and 82% at doses of 1, 3, 10, 30 and 100 mg/kg, respectively, compared to the control group (Figure 1B). The maximum percentage of gastroprotection obtained with 100 mg/kg of caryophyllene oxide was $77.0 \pm 9.3\%$, while that observed with 100 mg/kg of carbenoxolone was $82.5 \pm 4.3\%$.

Effect of L-NAME, indomethacin and NEM on the gastroprotective effect

The ulcer index of rats pretreated with 70 mg/kg L-NAME ($121.8 \pm 4.0 \text{ mm}^2$, Figure 2A), 10 mg/kg indomethacin ($122.7 \pm 8.1 \text{ mm}^2$, Figure 2B), or 10 mg/kg NEM ($130.3 \pm 7.9 \text{ mm}^2$, Figure 2C) was significantly different ($P < 0.05$) compared with the control animals treated only with saline solution ($94.1 \pm 9.8 \text{ mm}^2$). Previously, it was reported that these doses of inhibitors were enough to block NO synthase, prostaglandin synthesis and endogenous sulfhydryls, respectively (Arrieta *et al.*, 2003). Pretreatment with L-NAME (70 mg/kg, s. c.) attenuated the gastroprotective effect of both caryophyllene oxide (100 mg/kg) and carbenoxolone (100 mg/kg). The ulcer index obtained in L-NAME pretreated rats later treated with caryophyllene oxide ($118.1 \pm 10.0 \text{ mm}^2$) was not significantly different ($P < 0.05$) from that obtained with the L-NAME-pretreated control animals ($121.8 \pm 4.0 \text{ mm}^2$).

However, the ulcer index obtained in L-NAME pretreated rats later treated with carbenoxolone ($84.6 \pm 7.6 \text{ mm}^2$) was indeed significantly different than L-NAME pretreated control, but not different in respect to the vehicle control ($94.1 \pm 9.8 \text{ mm}^2$).

Pretreatment with indomethacin (10 mg/kg) also attenuated the gastroprotective effect of both caryophyllene oxide (100 mg/kg) and carbenoxolone (100 mg/kg). The maximum ulcer index obtained for indomethacin pretreated rats later treated caryophyllene oxide was $117.8 \pm 8.7 \text{ mm}^2$, which was not significantly different ($P < 0.05$) from that of the indomethacin pretreated control ($122.7 \pm 8.1 \text{ mm}^2$). The value of the ulcer index obtained with 100 mg/kg carbenoxolone after indomethacin pretreatment ($80.7 \pm 3.9 \text{ mm}^2$) was not significantly different ($P < 0.05$) from that of the vehicle control (Figure 2A).

Pretreatment with NEM (10 mg/kg) also attenuated the gastroprotective effect of both caryophyllene oxide (100 mg/kg) and carbenoxolone (100 mg/kg). Oral administration of caryophyllene oxide to NEM-pretreated (10 mg/kg) rats did not inhibit the ethanol-induced gastric lesions. In this case, the ulcer index obtained with NEM-pretreated rats later treated with caryophyllene oxide was $117.6 \pm 7.1 \text{ mm}^2$, a value not significantly different ($P < 0.05$) from that of the NEM-pretreated control ($130.3 \pm 7.9 \text{ mm}^2$). There was also no statistically significant difference in the inhibition of ethanol-induced gastric lesions with carbenoxolone treatment after pretreatment with NEM ($82.08 \pm 8.3 \text{ mm}^2$) when compared with the vehicle control.

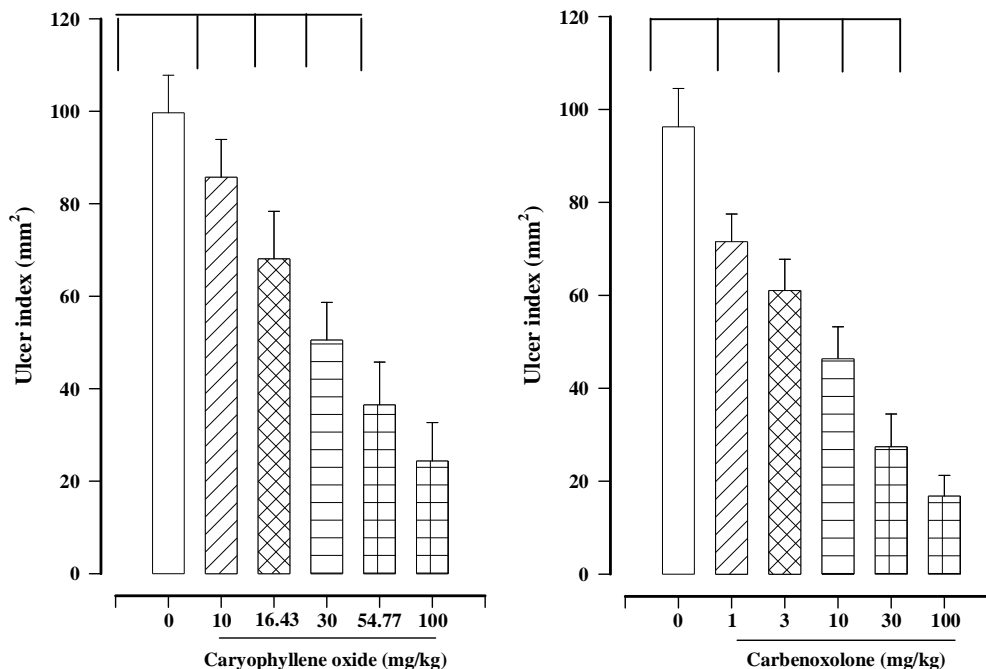


Fig. 1: Effect of different doses of (A) caryophyllene oxide (10-100 mg kg⁻¹) and (B) carbenoxolone (1-100 mg kg⁻¹) on gastric lesions induced in rats by absolute ethanol. Bars represent the mean \pm SEM (n=7-10). * $p < 0.05$, vs the respective control, according to Dunn's multiple comparison test after the Kruskal-Wallis test.

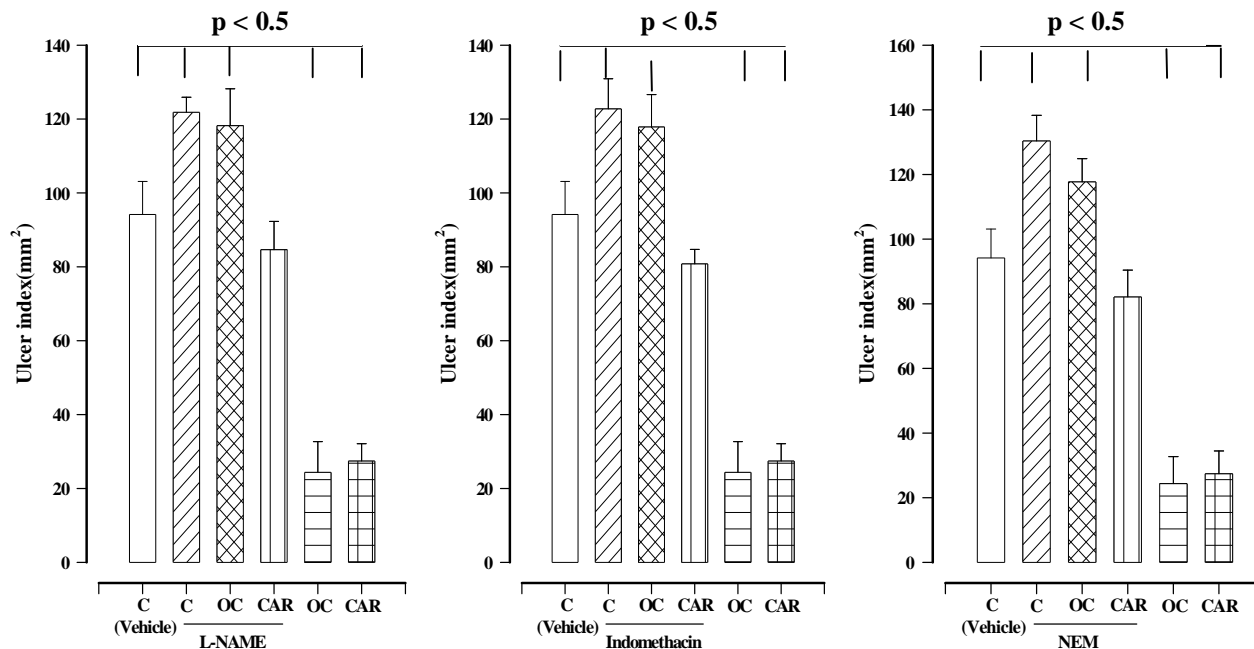


Fig. 2: Effect of caryophyllene oxide (OC) and carbenoxolone (CAR) at 30 mg kg⁻¹ on gastric lesions induced by ethanol in rats pretreated with (A) L-NAME (70 mg kg⁻¹), (B) indomethacin (10 mg kg⁻¹) or (C) NEM (10 mg kg⁻¹). Bars represent the mean \pm SEM (n=7-10). *p< 0.05, vs the respective control, according to Dunn's multiple comparison test after the Kruskal-Wallis test.

DISCUSSION

The present study tested the gastroprotective activity of caryophyllene oxide in the rat model of an ethanol-induced ulcer. Oral administration of both caryophyllene oxide (the test compound) carbenoxolone (the reference drug) reduced gastric lesions in a dose-dependent manner. This is the first report of the gastroprotective activity of caryophyllene oxide, which reached the maximum effect of $77.0 \pm 9.3\%$ at a dose of 100 mg/kg. The maximal gastroprotective effect induced by carbenoxolone was $82.5 \pm 4.3\%$, also at a dose of 100 mg/kg. Hence, these two values of maximum effect (at the same dose) are very similar, suggesting that both compounds have an equivalent efficacy. Considering that the prolonged use of NSAIDs can lead gastrointestinal damage, and that caryophyllene oxide has been elicited anti-inflammatory and analgesic activities, and in this work furthermore has demonstrated gastroprotective activity that might be advantageous compared to NSAIDs, because if it will use as anti-inflammatory or analgesic agent cannot lead gastric lesions.

The chemistry of caryophyllene is dominated by the flexible conformations of the nine-membered ring and the consequent relative positions of the reactive functional groups. Thus, the exploration of the structural/biological activity relationships in this family of natural products should certainly prove useful (Collado *et al.*, 1998). Interestingly, β -caryophyllene also has elicited anti-inflammatory and cytoprotective effects (Fernandes *et al.*, 2007).

Gastric ulcers are generally caused by a disruption in the balance between aggressive factors and mucosal defensive factors. Agents that present gastroprotection against ethanol-induced gastric

Lesions act mainly by stimulation of defense mechanisms (cytoprotective effect) rather than inhibition of the aggressive factor that produces or releases molecules with an antisecretory effect. Therefore, in the present study we investigated the mechanism of action of caryophyllene oxide in relation to some gastric mucosa protective factors, such as nitric oxide (NO), prostaglandins and sulfhydryl compounds.

The mechanism of action of some gastroprotector compounds obtained from medicinal plants involve NO (5). It is well recognized that NO is involved in the modulation of acid, alkaline and mucus secretion, gastric mucosal blood flow, and the reduction of lipid peroxidation (Ancha *et al.*, 2003). Our results show an increase in gastric lesions when blocking NO production with L-NAME pretreatment before caryophyllene oxide treatment, suggesting that the gastroprotective effect of the test compound is mediated by the NO pathway (Figure 2A).

On the other hand, gastric mucosal integrity requires the continuous generation of prostaglandin E₂ (PGE₂), which is crucial for protection against ulcerogenic and necrotizing agents (Chandranath *et al.*, 2002). Almost all mucosal defense mechanisms are stimulated and/or facilitated by prostaglandins (PGs). Our data suggest that the cytoprotective action of caryophyllene oxide on the gastric mucosa is related with PGs, evidenced by the fact that pretreatment with indomethacin (a COX inhibitor) inhibited the gastroprotection exerted by caryophyllene oxide (Figure 2B).

Recent research has also highlighted the fact that the protective functions of prostaglandins in the stomach can be exerted by other mediators, such as NO. For instance, it has been

shown that NO released from constitutive NOS activates COX-1 (Khattab *et al.*, 2001), which in turn promotes gastric mucosal protection (Cuzzocrea and Salvemini, 2007). Hence, the results suggest that caryophyllene oxide could stimulate a cross-talk between NO and PGs. Further studies are needed to explore this possibility. The results obtained with carbenoxolone (Figure 2A and B) indicate that NO and PGs are implicated in the mechanism of action of this reference drug as well, which is in accordance with reports in the literature (Sánchez-Mendoza *et al.*, 2011).

Endogenous sulfhydryl compounds help maintain the integrity of the mucus barrier by uniting its subunits through disulfide bridges, thus preventing the mucus from becoming soluble and easily withdrawn by ulcerogenic agents, such as ethanol (Gupta and Eisen, 2009). Sulfhydryl compounds also prevent the production of free radicals by ethanol and act as recycling antioxidants (Avila *et al.*, 1996). The rats that were pretreated with the inhibitor of sulfhydryl compounds before treatment with caryophyllene oxide presented an ulcer index similar to animals treated only with NEM, which indicates the importance of an intact sulfhydryl barrier in the maintenance of the gastroprotective effect of caryophyllene oxide. Regarding carbenoxolone, the results obtained with NEM are in agreement with the literature (Sánchez-Mendoza *et al.*, 2011).

CONCLUSION

In the current contribution the gastroprotective activity of caryophyllene oxide was demonstrated. Endogenous NO, prostaglandins and sulfhydryl groups all seem to play an important role in the gastroprotective mechanism of caryophyllene oxide on ethanol-induced gastric lesions.

ACKNOWLEDGMENTS

This work was supported by two grants from the Escuela Superior de Medicina of the Instituto Politécnico Nacional, México (SIP 20140951 and SIP 20140989).

REFERENCES

- Ancha H, Ojeas H, Tedesco D, Ward A, Harty RF. Somatostatin-induced gastric protection against ethanol: Involvement of nitric oxide and effects on gastric mucosal blood flow. *Regul Pept*, 2003; 110: 107–113.
- Arrieta J, Benitez J, Flores E, Castillo C, Navarrete A. Purification of gastroprotective triterpenoids from the stem bark of *Amphipterygium adstringens*; role of prostaglandins, sulfhydryls, nitric oxide and capsaicin-sensitive neurons. *Planta Med*, 2003; 69: 905–909.
- Avila JR, de la Lastra CA, Martin MJ, Motilva V, Luque I, Delgado D, Esteban J, Herrerias J. Role of endogenous sulphhydryls and neutrophil infiltration in the pathogenesis of gastric mucosal injury induced by piroxicam in rats. *Inflamm Res*, 1996; 45: 83–88.
- Banerjee D, Maity B, Nag SK, Bandyopadhyay SK, Chattopadhyay S. Healing potential of *Picrorhiza kurroa* (Scrophulariaceae) rhizomes against indomethacin-induced gastric ulceration: a mechanistic exploration. *BMC Complement Altern Med*, 2008; 8: 3.

Collado IG, Hanson JR, Macías-Sánchez AJ. Recent advances in the chemistry of caryophyllene. *Nat Prod Rep*, 1998; 15: 187–204.

Cuzzocrea S, Salvemini D. Molecular mechanisms involved in the reciprocal regulation of cyclooxygenase and nitric oxide synthase enzymes. *Kidney Int*, 2007; 71: 290–297.

Chandranath SI, Bastaki SM, Singh J. A comparative study on the activity of lansoprazole, omeprazole and PD-136450 on acidified ethanol-and indomethacin-induced gastric lesions in the rat. *Clin Exp Pharmacol Physiol*, 2002; 29: 173–180.

Chavan MJ, Wakte PS, Shinde DB. Analgesic and anti-inflammatory activity of caryophyllene oxide from *Annona squamosa* L. bark. *Phytomedicine*, 2010; 17: 149–151.

Fernandes ES, Passos GF, Medeiros R, da Cunha FM, Ferreira J, Campos MM, Pianowski LF, Calixto JB. Anti-inflammatory effects of compounds alpha-humulene and (–)-trans-caryophyllene isolated from the essential oil of *Cordia verbenacea*. *Eur J Pharmacol*, 2007; 569: 228–236.

Gupta M, Eisen GM. NSAIDs and the gastrointestinal tract. *Curr Gastroenterol Rep*, 2009; 11: 345–353.

Jainu M, Devi CS. Antiulcerogenic and ulcer healing effects of *Solanum nigrum* (L.) on experimental ulcer models: Possible mechanism for the inhibition of acid formation. *J Ethnopharmacol*, 2006; 104: 156–163.

Jun NJ, Mosaddik A, Moon JY, Jang KC, Lee DS, Ahn, KS, Cho SK. Cytotoxic activity of β-caryophyllene oxide isolated from Jeju guava (*Psidium cattleianum* Sabine) leaf. *Rec Nat Prod*, 2011; 5: 242–246.

Khattab MM, Gad MZ, Abdallah D. Protective role of nitric oxide in indomethacin induced gastric ulceration by a mechanism independent of gastric acid secretion. *Pharmacol Res*, 2001; 43: 463–467.

Polanco-Hernández G, Escalante-Erosa F, García-Sosa K, Rosado ME, Guzmán-Marín E, Acosta-Viana KY, Giménez-Turba A, Salamanca E, Peña-Rodríguez LM. Synergistic Effect of Lupenone and Caryophyllene Oxide against *Trypanosoma cruzi*. *Evid Based Complement Alternat Med*, 2013; 2013: 435398.

Sánchez-Mendoza ME, Reyes-Ramírez A, Cruz Antonio L, Martínez Jiménez L, Rodríguez-Silverio J, Arrieta J. Bioassay-guided isolation of an anti-ulcer compound, tagitinin C, from *Tithonia diversifolia*: Role of nitric oxide, prostaglandins and sulfhydryls. *Molecules*, 2011; 16: 665–674.

Toma W, Hiruma-Lima CA, Guerrero RO, Brito AR. Preliminary studies of *Mammea americana* L. (Guttiferae) bark/latex extract point to an effective antiulcer effect on gastric ulcer models in mice. *Phytomedicine*, 2005; 12: 345–350.

Vera-Arzave C, Antonio L, Arrieta J, Cruz-Hernández G, Velázquez-Méndez AM, Reyes-Ramírez A, Sánchez-Mendoza ME. Gastroprotection of suaveolol, isolated from *Hyptis suaveolens*, against ethanol-Induced gastric lesions in wistar rats: role of prostaglandins, nitric oxide and sulfhydryls. *Molecules*, 2012; 17: 8917–8927.

Vonkeman HE, Klok RM, Postma MJ, Brouwers JR, van de Laar MA. Direct medical costs of serious gastrointestinal ulcers among users of NSAIDs. *Drugs Aging*, 2007; 2: 681–690.

Yeomans ND, Naesdal J. Systematic review: ulcer definition in NSAID ulcer prevention trials. *Aliment Pharmacol Ther*, 2008; 27:465–472.

How to cite this article:

María Elena Sánchez-Mendoza, Leticia Cruz-Antonio, María Guadalupe Cupido-Sánchez, Guillermo García-Castillo, Jesús Arrieta. Gastroprotective activity of caryophyllene oxide: the role of nitric oxide, prostaglandins and sulfhydryls. *J App Pharm Sci*, 2014; 4 (09): 001-005.