

## Gastroprotective effects of D-002 (beeswax alcohols) and Lyprinol® on experimentally-induced gastric ulceration in rats

## Efectos gastroprotectores del D-002 (alcoholes de cera de abejas) y el Lyprinol® sobre la úlcera gástrica experimental en ratas

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### ABSTRACT

**Introduction:** D-002, a mixture of beeswax alcohols, has been effective in osteoarthritis models and for reducing osteoarthritis symptoms. Unlike the classic anti-inflammatory drugs, D-002 elicits gastroprotective rather than gastrototoxic effects. Lyprinol, used for ameliorating inflammation and arthritic symptoms, improves gastrointestinal dysfunction symptoms in osteoarthritis subjects. Both D-002 and Lyprinol inhibit cyclooxygenase and 5-lipoxygenase activities, and have been similarly effective for reducing inflammation experimentally.

**Objective:** to compare the effects of D-002 and Lyprinol on gastric mucosa of normal and experimentally-induced ulcer rats.

**Methods:** ulcer indexes were measured in normal rats and in rats with ethanol or pylorus ligation-induced ulcers, in which gastric volume and mucus secretion were also measured. Normal rats were randomized into a vehicle control, one acetic salicylic acid (150 mg/kg), three D-002, three Lyprinol groups; rats with ethanol-ulcers into a vehicle control, three D-002 and three Lyprinol-treated groups; and the experiment on pylorus ligation included a negative control and eight pylorus-ligated groups: one vehicle control, three D-002, three Lyprinol, one omeprazole 10 mg/kg. In all cases, D-002 and Lyprinol (50, 200 and 400 mg/kg) were given orally.

**Results:** unlike D-002 and Lyprinol (50-400 mg/kg), acetic salicylic acid increased

ulcer indexes and the incidence of ulcers versus the vehicle control. Single oral doses of D-002 (50-400 mg/kg) or Lyprinol (200 and 400 mg/kg) decreased significantly ( $p < 0.01$ ) and in a similar way ulcer indexes versus the ethanol-positive control. D-002 and Lyprinol (50-400 mg/kg) lowered significantly ( $p < 0.01$ ) and comparably ulcer indexes in rats with pylorus ligation versus the positive controls. D-002 (200 and 400 mg/kg) decreased gastric volume and increased gastric mucus secretion versus the positive control whereas only Lyprinol 400 mg/kg increased the gastric mucus secretion but without modifying the gastric volume. Omeprazole significantly reduced ulcer index ( $p < 0.05$ ) and gastric volume ( $p < 0.01$ ), with no change in mucus secretion.

**Conclusion:** D-002 and Lyprinol did not show gastrototoxic effects and similar efficacy in protecting against ethanol and pylorus ligation-induced gastric ulceration in rats

**Keywords:** D-002, Lyprinol, ethanol ulcers, pylorus ligation, gastroprotection.

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## RESUMEN

**Introducción:** el D-002, una mezcla de alcoholes de la cera de abejas, efectivo en modelos de osteoartritis y para reducir los síntomas de la misma. A diferencia de los medicamentos antiinflamatorios clásicos el D-002 produce efectos gastroprotectores más que efectos gastrotóxicos. El Lyprinol, usado para disminuir la inflamación y los síntomas artríticos, mejora los síntomas de disfunción gastrointestinal en sujetos con dicha enfermedad. D-002 y Lyprinol inhiben las actividades de ciclooxigenasa y 5-lipooxigenasa, y son similarmente efectivos para reducir la inflamación en modelos experimentales.

**Objetivo:** comparar los efectos del D-002 y el Lyprinol sobre la mucosa gástrica de ratas normales y de ratas con úlcera gástrica inducida experimentalmente.

**Métodos:** se determinó el índice de úlcera en ratas normales y en ratas con úlceras gástricas inducidas por etanol e inducidas por ligadura de píloro, en las cuales se midió el volumen gástrico y la secreción de mucus. Las ratas normales se distribuyeron en un grupo control (vehículo), uno con ácido acetil salicílico (150 mg/kg), tres con D-002 y tres con Lyprinol; las ratas con úlcera inducida por etanol en un grupo control (vehículo), tres con D-002 y tres con Lyprinol; y el experimento con ligadura de píloro en un grupo control (vehículo), tres D-002, tres Lyprinol y uno con omeprazol (10 mg/kg). En todos los casos, el D-002 y el Lyprinol (50, 200 y 400 mg/kg) se administraron por vía oral.

**Resultados:** el ácido acetil salicílico, no el D-002 ni el Lyprinol (50-400 mg/kg), incrementó el índice de úlceras y la incidencia de úlceras comparadas con el grupo control. Dosis orales únicas de D-002 (50-400 mg/kg) o Lyprinol (200 y 400 mg/kg) redujeron significativa ( $p < 0,01$ ) y similarmente el índice de úlceras comparado con el grupo control positivo con úlceras por etanol. El D-002 y el Lyprinol (50-400 mg/kg) redujeron significativamente ( $p < 0,01$ ) y comparablemente el índice de úlceras en ratas con ligadura de píloro comparado con el grupo control positivo. El D-002 (200 y 400 mg/kg) redujo el volumen gástrico e incrementó la secreción de mucus gástrico respecto al grupo control positivo; mientras solo el Lyprinol 400 mg/kg aumentó la secreción de mucus gástrico pero sin modificar el volumen gástrico. El omeprazol redujo significativamente el índice de úlcera ( $p < 0,05$ ) y el volumen gástrico ( $p < 0,01$ ), sin modificar la secreción de mucus.

**Conclusiones:** el D-002 y el Lyprinol no presentaron efectos gastrotóxicos, y protegieron con eficacia similar de las úlceras gástricas inducidas por etanol y por ligadura del píloro en ratas.

**Palabras clave:** D-002, Lyprinol, úlcera gástricas por etanol, ligadura del píloro, gastroprotección.

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## INTRODUCTION

Inflammation is a normal host response against deleterious stimuli. Acute inflammation involves short-term responses to pro-inflammatory injuries and further repair, but if this mechanism fail it may evolves to chronic inflammation, which has been linked with long-standing diseases, including osteoarthritis.<sup>1</sup> The inflammatory response involves the increased production of arachidonic acid from membrane phospholipids, which is then metabolized by cyclooxygenase (COX) and lipoxygenase (LOX) pathways to produce prostaglandins (PG), thromboxanes, prostacyclins, and leukotrienes (LT).<sup>2</sup>

Currently non selective non-steroidal anti-inflammatory drugs (NSAIDs) and COX-2 inhibitors, very effective to treat inflammation and associated pain, are among the most prescribed drugs worldwide. Basically, NSAIDs and COX-2 inhibitors display their anti-inflammatory effects by inhibiting the COX pathway, but such a mechanism also supports some of their relevant drug-related adverse effects, like gastric damage and bronchospasm linked with COX-1 inhibition, and cardiovascular adverse side effects caused by COX2 inhibition.<sup>3</sup> NSAIDs-induced gastric damage, a relative limitation for their clinical use, results from the curtailed production of gastroprotective PG and the displacement of the arachidonic acid metabolism towards the LOX pathway, which increases the synthesis of pro-inflammatory and gastrototoxic LT, so that increased concentrations of LTB<sub>4</sub> have been found on the walls of NSAID-induced gastric ulcers, which attract leukocytes and contribute to cause stomach ulceration,<sup>4</sup> enhancing the gastrototoxicity due to the PG deficit.<sup>3</sup>

Keeping in mind the extended use of NSAIDs for vascular prevention, which is the case for acetic salicylic acid (ASA), and inflammation-related diseases, like osteoarthritis, the incidence of NSAIDs-related gastric damage and complications have been increasing, a trends predicted to continue due the increase on life expectancy, so that special recommendations for their use in the elderly have been reported.<sup>5</sup> This background supports the search of effective and safer anti-inflammatory substances, like dual inhibitors of COX and 5-LOX, which cut PG synthesis and provide anti-inflammatory effects, but also prevent the switch to an increased LT production by inhibiting 5-LOX, thus reducing the occurrence of gastric adverse effects.<sup>6</sup> The inhibition of LTB<sub>4</sub> synthesis, therefore, seems to be a relevant target of anti-inflammatory drugs devoid of gastrototoxicity.<sup>7</sup>

D-002, a mixture of six beeswax alcohols with triacontanol as the main component, has been effective for lowering experimental inflammation<sup>8</sup> and OA symptoms.<sup>9</sup> D-002 was also found to inhibit both COX and 5-LOX activity,<sup>10</sup> and to lower LTB<sub>4</sub> levels in the pleural exudates of rats with carrageenan-induced pleurisy.<sup>7</sup> Unlike the classic anti-inflammatory drugs, however, D-002 has shown to cause gastroprotective, rather than gastrotoxic effects, in experimental<sup>11,12</sup> and clinical studies.<sup>13</sup> Although the dual inhibition of COX and 5-LOX may support the lack of D-002 gastrototoxicity; this mechanism alone cannot explain the gastroprotective

effect of D-002, which involves antioxidant effects on the gastric mucosa and increased gastric mucus secretion.<sup>11,12</sup>

Lyprinol, trade mark of a lipid extract from green-lipped mussel (GLM) (*Perna canaliculus*), inhibits COX and 5-LOX enzymes and reduces the production of LTB<sub>4</sub>. Lyprinol has been used for the symptomatic relief of inflammatory conditions, including arthritis, not only without causing the adverse effects of NSAIDs, but even improving symptoms of gastrointestinal dysfunction in OA subjects.<sup>14</sup>

A previous study found that D-002 and Lyprinol were similarly effective for lowering the oedema in the model of carrageenan-induced pleurisy,<sup>15</sup> but their effects on gastric ulceration had not been compared yet.

This study compares the effects of single oral doses of D-002 and Lyprinol on gastric ulceration in rats.

## METHODS

### ANIMALS

Male *Sprague Dawley* rats (250-300g) from the National Center for Laboratory Animal Production (CENPALAB, Havana, Cuba) were used for the study and adapted for 7 days to the following conditions: temperature (22-23 °C), relative humidity (55-60 %) and 12 hours dark/light cycles. Food and water were freely supplied. The animals had fasted for 24 h prior to the experiments.

The experiments were performed in accordance with the care and use of experimental animals prescribed by the Cuban Guidelines for the care of laboratory animals and the Cuban Code of Good Laboratory Practice (GLP), after obtaining the approval of the Institutional Board for animal use. The study protocol and animals use were approved prior to the study by the Institutional Animal Ethics Committee (No 035/2011).

### ADMINISTRATION AND DOSAGE

The batch of D-002, supplied by the Plants of Natural Products (National Center for Scientific Research, Havana City, Cuba) was used after corroborate its quality criteria. Batch composition, assessed with a validated gas chromatographic method,<sup>16</sup> was as follows: 1-tetracosanol (5 %), 1-hexacosanol (10.2 %), 1-octacosanol (14 %), 1-triacontanol (34.21 %), 1-dotriacontanol (24.24 %) and 1-tetratriacontanol (3.03 %). Purity (total content of these alcohols) was 90.7 %.

Lyprinol soft gel capsules (Blackmores, Australia, batch: 252918) used for the experiments contained 50 mg of the lipid extract from GLM diluted in olive oil and Vitamin E. GLM contain free fatty acids, mainly omega-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (18 %) and docosahexanoic acid (15%), palmitic acid (16 %), cholesterol ( $\approx$ 30 %), sterol esters, triglycerides, and polar lipids, as reported.<sup>17</sup> The content of the capsules was directly drawn with a syringe and used in the experiments.

Omeprazole (lote OE513187) from Laboratorios NOVATEC (La Habana, Cuba), was dissolved in acacia gum/water vehicle (10 mg/mL) and then used in the experiments. D-002 and Lyprinol were suspended in 2 % Tween 65/water,

respectively, while ASA (from the Cuban Pharmaceutical Industry, Havana, Cuba) was dissolved in acacia gum/water vehicle (10 mg/mL). All treatments (vehicle, D-002, Lyprinol, Aspirin and Omeprazole) were administered orally by gastric gavage (5mL/kg).

#### EXPERIMENTAL DESIGN

Three experiments were performed for comparing the effects of single oral doses of D-002 and Lyprinol on gastric ulceration in rats. The first experiment investigated the potential gastro-toxic effects of D-002 and Lyprinol in normal rats, while the two other series compared the effects of both substances on ethanol and pylorus ligation-induced ulcers in rats by assessing their effects on ulcer index.

#### EFFECTS ON THE GASTRIC MUCOSA OF NORMAL RATS

Rats were randomized into eight groups (10 rats per group): a vehicle control, three treated with D-002 (50, 200 and 400 mg/kg), three treated with Lyprinol (50, 200 and 400 mg/kg) and one with aspirin (150 mg/kg).

All treatments were administered for seven days. After the last administration, rats were fasted for 24 h with free access to water and then they were sacrificed by using an overdose of thiopental anaesthesia (80 mg/kg). The stomachs were immediately removed for quantifying the lesions.

#### EFFECTS ON ETHANOL INDUCED GASTRIC ULCERS IN RATS

Seven groups of 10 rats each one were treated with the control vehicle; D-002 (50, 200 and 400 mg/kg) or Lyprinol (50, 200 and 400 mg/kg) one hour prior to oral administration of ethanol (60 % ethanol; 1 mL/200 g).<sup>18</sup> The animals were sacrificed after one hour under an overdose of thiopental anesthesia and their stomachs were washed with cold saline and removed for examining the ulcer index microscopically.

#### EFFECTS ON PYLORUS LIGATION INDUCED GASTRIC ULCERS IN RATS

Rats were randomized into nine groups of 10 rats each one, a negative control and eight pylorus-ligated groups that were orally administered with the vehicle (positive control), D-002 (50, 200 and 400 mg/kg), Lyprinol (50, 200 and 400 mg/kg) or omeprazole 10 mg/kg, respectively.

After one hour, the rats were anaesthetized with thiopental (40 mg/kg, i.p), the abdomen was cut open, the pyloric end was ligated without damaging the blood supply and the abdominal cavity was closed. The rats were anaesthetized and sacrificed four hours after pylorus ligation, the stomachs were removed and opened along the greater curvature and the ulcer indexes were measured. The volume of gastric juice, the mucus weight/stomach weight ratio and the concentrations of total hydroxyperoxides (THP) in gastric mucus were also measured.

#### EVALUATION OF GASTRIC ULCERATION

The lesions in the gastric mucosa were examined macroscopically using magnification 3x. Ulcer indexes were determined as the sum of the lengths of the whole gastric lesions (in mm). Two independent, blinded observers performed the observations and measurements of lesion lengths.<sup>19</sup>

#### GASTRIC JUICE VOLUME

The gastric contents were collected and centrifuged at 3000 rpm<sup>-1</sup> for 10 min. The supernatant was used for determining the gastric volume.

#### MUCUS WEIGHT/STOMACH WEIGHT RATIO

Stomachs were weighed in *Mettler* Toledo balance, opened along the greater curvature and then the mucus was scraped gently with a scalpel and weighed immediately in the *Mettler* Toledo balance (model: AG204, manufacturer: *DeltaRange*<sup>®</sup>, precision: 0.0001g). The amount of mucus was expressed as the mucus weight/stomach weight ratio.

#### MEASUREMENT OF LIPID PEROXIDATION

The stomachs were washed with normal saline, cut into small pieces and homogenized in a Potter-Elvehjem glass homogenizer in ice cold 0.15 M KCl to obtain 20 % homogenates used for the determination of THP,<sup>20</sup> a marker of lipid peroxidation. In brief 0.1 mL of the homogenate was allowed to react with 0.9 mL of Fox reactive (88 mg of butylated hydroxytoluene, 7.6 mg of orange xyleneol, 9.8 mg of ammonium sulphate were dissolved in a 9:1 mixture of methanol:sulphuric acid). This mixture was incubated at 37 °C for 30 min and then cooled. This mixture was centrifuged at 2400xg for 10 min and the absorbance of the supernatant was measured at 560 nm. The concentrations of THP were calculated from a standard calibration curve generated with cumene hydroperoxide. THP values were expressed as mol/mg of tissue protein.

#### STATISTICAL ANALYSES

Comparisons among groups were done with the *Kruskal Wallis test*, paired comparisons between each treated and control groups with the *Mann-Whitney U test* and categorical data (ulcer frequency) with the *Fisher's Exact Probability test*. Statistical significance was chosen for  $\alpha=0.05$ . Data were processed with the *Statistics Software for Windows* (Release 6.1 Stat Soft Inc, Tulsa OK, USA).

## RESULTS

#### EFFECTS ON THE GASTRIC MUCOSA OF NORMAL RATS

No rat from the vehicle control group, but all treated with aspirin 150 mg/kg exhibited gastric ulceration, so that both the ulcer index and the frequency of rats with gastric ulcers were significantly higher in the aspirin group as compared to the vehicle control. By contrast, no rat treated with D-002 (50-400 mg/kg), and only two treated with Lyprinol at 200 and 400 mg/kg, respectively, displayed gastric ulcers. In consequence, ulcer index and frequency of animals with gastric ulceration in these two groups were similar to those of the control group (Table 1).

**Table 1.** Effects of treatments on normal gastric mucosa in rats

Treatments	Ulcer index (mm)	Ulcer frequency (%)
Vehicle (control)	0±0	0/8 (0 %)
Aspirin 150 mg/kg	16,80±4.20*	10/10 (100 %) <sup>§</sup>
D-002 50 mg/kg	0±0	0/8 (0 %)
D-002 200 mg/kg	0±0	0/8 (0 %)
D-002 400 mg/kg	0±0	0/8 (0 %)
Lyprinol 50 mg/kg	0±0	0/8 (0 %)
Lyprinol 200 mg/kg	0,05±0,05	1/8 (12 %)
Lyprinol 400 mg/kg	0,31±0,31	1/8 (12 %)

\* p<0.001 Comparison with the control; (Mann Whitney U test)  
<sup>§</sup>p<0.001 Comparison with the control; \* p<0.001 Comparison with D-002; Comparison with Lyprinol (Fisher's Exact probability test)

### EFFECTS ON ETHANOL-INDUCED GASTRIC ULCERS

Pre-treatment of rats with single oral doses of D-002 (50-400 mg/kg) decreased significantly and markedly (38.8% to 71.0 %) the ulcer index induced by ethanol as compared to the positive control (table 2). In turn, Lyprinol 200 and 400 mg/kg, not 50 mg/kg, reduced significantly and markedly (67.5 % and 73.7 %) the ulcer index in this model. The reductions of ulcer index with both substances were similar.

**Table 2.** Effects of treatments on ethanol-induced ulceration in rats

Treatments	Ulcer index (mm)	I (%)
Vehicle control	48,60±8,90	—
D-002 50 mg/kg	29,74±7,20*	38,8
D-002 200 mg/kg	17,80±6,00*	63,4
D-002 400 mg/kg	14,10±4,00**	71,0
Lyprinol 50 mg/kg	40,38±5,10	16,9
Lyprinol 200 mg/kg	15,80±5,40**	67,5
Lyprinol 400 mg/kg	12,80±2,70**	73,7

I % percent inhibition,  
 \*p<0.05, \*\*p<0.01, Comparison with the control (Mann Whitney U test)

### EFFECTS ON PYLORUS LIGATION INDUCED GASTRIC ULCERS

Table 3 summarizes the effects of treatments on pylorus ligation-induced gastric damage in rats. Oral acute administration of D-002 (50-400 mg/kg) produced significant decreases (56 -63 %) of the ulcer index as compared with the positive control group, and statistically similar reductions (52- 64 %) were found with Lyprinol (50-400 mg/kg) (table 3). Also, all doses of both substances (50, 200 and 400 mg/kg) reduced significantly THP concentrations in the mucus (44, 59 and 63 % with D-002; respectively; 22, 34 and 40 % with Lyprinol, respectively), but D-002 was more effective than Lyprinol for lowering this variable across all the dose range. Also, D-002 (200 and 400 mg/kg) decreased the gastric volume (34 and 39 %, respectively) and increased gastric mucus secretion versus the positive control; meanwhile only the highest dose of Lyprinol (400 mg/kg) increased significantly the gastric mucus secretion but without modifying the gastric

volume. Omeprazole 10 mg/kg significantly reduced ulcer index (60 %), gastric volume (50 %) and mucus THP values (93 %), but unchanged mucus secretion.

**Table 3.** Effects of treatments on pylorus ligation induced gastric ulcers in rats

Treatments	Ulcer index (mm)	I (%)	Volume of gastric secretion (mL)	I (%)	Mucus weight/stomach weight ratio	THP (mol/mg of tissue protein)	I (%)
Vehicle +not pylorus-ligation (sham)	0 ± 0*	—	0,1 ± 0,3***	—	92,9 ± 5,8	10,3 ± 0,6***	—
Pylorus ligation +							
Vehicle (positive control)	19,7 ± 3,2	—	10,4 ± 1,5	—	86,7 ± 2,1	29,2 ± 2,3	—
D-002 50 mg/kg	8,6 ± 1,0**	56	8,6 ± 0,6	17	92,8 ± 8,3	20,9 ± 1,0*	44
D-002 200 mg/kg	7,3 ± 1,0**	63	6,9 ± 0,7 <sup>†</sup>	34	107,5 ± 5,2***	18,0 ± 6,4**	59
D-002 400 mg/kg	7,2 ± 0,9**	63	6,4 ± 0,8**	39	106,7 ± 7,0*	17,2 ± 1,2**	63
Lyprinol 50 mg/kg	9,5 ± 3,1*	52	8,6 ± 1,6	17	88,4 ± 3,5	25,0 ± 1,0* ++	22
Lyprinol 200 mg/kg	8,7 ± 2,7*	56	8,6 ± 0,5	17	85,9 ± 3,3	22,8 ± 0,6***	34
Lyprinol 400 mg/kg	7,0 ± 1,3**	64	9,0 ± 0,8	14	96,2 ± 2,6 <sup>†</sup>	21,6 ± 0,6***	40
Omeprazole 10 mg/kg	7,9 ± 2,6*	60	5,1 ± 0,8**	50	86,0 ± 4,8	11,6 ± 1,6***	93

I % percent inhibition, THP total hydroxyperoxides;  
<sup>†</sup>p<0.05; \*\*p<0.01; \*\*\*p<0.001 Comparison with the positive control;  
<sup>†</sup>p<0.05; \*\*p<0.01 Comparison with D-002 (Mann Whitney U test)

## DISCUSSION

Inflammation of the gastric mucosa may affect its own defensive protection, which may lead to gastric mucosal ulceration. Effective management of gastric ulceration, which results from the imbalance between gastroprotective and aggressive factors, would primarily depends on the reduction of the aggressive factors, the reinforcement of protective factors or a combination of both.<sup>21</sup>

Gastric ulcers may arise frequently due to the chronic use of external aggressive factors, such as NSAIDs, stress, smoking, and alcohol consumption.<sup>21</sup> Nowadays, drug-induced damage to the gastrointestinal tract has become a health problem, in great part due to the extensive use of NSAIDs,<sup>3</sup> which could increase dramatically if its use for chemoprotection against different cancers is expanded.<sup>22</sup>

In this study, it was demonstrated that the repeated oral administration of D-002 or Lyprinol (50-400 mg/kg) did not induce gastric ulceration in rats, differently from aspirin (150 mg/kg), which produced evident ulcers in all treated rats, as expected. Beyond this lack of gastrototoxic effects caused by either D-002 or Lyprinol, both substances (50 – 400 mg/kg) demonstrated to be similarly effective for protecting against gastric ulceration induced by ethanol or pylorus ligation, this study being the first one reporting such effect for Lyprinol (*Entrez PubMed*, up to April 2014).

We found that the decrease of pylorus ligation-induced gastric ulceration produced by both D-002 and Lyprinol was accompanied by a reduction of lipid peroxidation (assessed by THP levels). The gastroprotective effect of D-002, not of Lyprinol, on pylorus ligation-induced ulceration also involves a decrease of gastric volume and an increase of gastric mucus secretion, in agreement with previous findings found in other ulcer models<sup>11</sup> and Omeprazole, a classic proton pump inhibitor used as reference drug, was able to reduce the ulcer index, the gastric volume, and THP concentrations, in agreement with its antisecretory, antiapoptotic and antioxidant

effects.<sup>23</sup> These results agree with those of other authors,<sup>24</sup> but diverge of those of pantoprazole, which was ineffective in this model.<sup>25</sup>

The gastric ulceration produced by pyloric ligation is caused by the increased accumulation of gastric acid and pepsin, leading to auto-digestion of gastric mucosa. Defensive factors like the mucus are known to protect the submucosal layers from the back-diffusion of hydrogen ions. Administration of D-002 and the highest doses of Lyprinol significantly increased mucus secretion, which accounts for a reinforcement of the defensive factors acting on the gastric mucosa. D-002 also significantly reduced the gastric volume. Both D-002 and Lyprinol significantly decreased the concentrations of THP in mucosa homogenates when compared to the control group of animals. The antioxidant effect of D-002 on the gastric mucosa is consistent with previous reports,<sup>12</sup> meanwhile the effect on lipid peroxidation of Lyprinol on this target is documented for the first time and must be corroborate for further studies. D-002, however, was more effective than Lyprinol regarding for lowering the extent of lipid peroxidation in gastric homogenates. The decrease on lipid peroxidation could play an important role in the protection of the gastric mucosa, as oxidative damage is accepted to be a common factor in the pathogenesis of gastric ulceration.<sup>26</sup>

Indeed, dual inhibition of D-002 and Lyprinol on COX and LOX pathways could be beneficial in limiting inflammation when compared to the conventional NSAIDs; and the joint blockade of both pathways may also lower the incidence of gastric ulceration.

## CONCLUSION

This study demonstrates that instead to be gastrototoxic, oral administration of D-002 and Lyprinol similarly protected against ethanol and pylorus ligation-induced gastric ulceration. In pylorus-ligated rats, D-002 and Lyprinol increased mucus secretion but D-002 displayed the greatest reduction of lipid peroxidation. Taken together, these data merit further research and enhance our understanding of the gastric safety of two dual inhibitors of COX and 5-LOX enzymes, namely D-002 and Lyprinol, which seem to reinforce some defensive factors on the gastric mucosa.

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