

## COMPARATIVE EFFECTS OF D-002, RANITIDINE AND OMEPRAZOLE ON ACETIC ACID-INDUCED ULCERS

DAISY CARBAJAL, VIVIAN MOLINA\*, MIRIAM NOA, YAZMIN RAVELO, ROSA MAS, MAIKEL VALLE

Department of Pharmacology. Center of Natural Product. National Center for Scientific Research, Havana, Cuba.

Email: vivian.molina@cnic.edu.cu

Received: 23 Aug 2013, Revised and Accepted: 14 Sep 2013

### ABSTRACT

D-002, a mixture of six higher aliphatic primary alcohols from beeswax, has been shown to produce gastroprotective effects mediated by increased gastric mucus secretion, improved mucus composition, and the reduction of lipid peroxidation. D-002 is able to heal acetic acid-induced gastric ulcers in rats, but its effects on this model had not been compared with those of proton pump inhibitors (PPI) or histamine 2-receptor (H2RA). This study compared the effects of D-002, omeprazole, and ranitidine on acetic acid-induced gastric ulcers in the rat, and on the associated neutrophil infiltration and angiogenesis in the ulcerated areas.

Rats were randomized into eight groups: a vehicle control and seven with acetic acid-induced ulceration: a positive control, two D-002 (200 and 400 mg/kg, respectively), two omeprazole (5 and 10 mg/kg), and two ranitidine (25 and 50 mg/kg) groups. Gastric ulcers were produced by serosal application of acetic acid. Ulcer indexes and histological assessment were done.

Significant reductions of ulcer indexes were seen with D-002 (200 and 400 mg/kg) (49% and 60%, respectively), omeprazole (5 and 10 mg/kg) (39% and 61%), and ranitidine (25 and 50 mg/kg) (52% and 68%). All treatments reduced ulcer sizes and inflammatory infiltrates, with signs of re-epithelization, both groups of D-002 and the highest dose of omeprazole showed the greatest effect on angiogenesis. Concluding, at the doses tested, D-002 healed acetic acid-induced ulcers as effectively as omeprazole and ranitidine, an effect associated to the reduction of neutrophil infiltration and to the increase of restorative angiogenesis into the ulcerated areas

**Keywords:** Acetic acid, D-002, Histology, Gastric ulcer, Omeprazole, Ranitidine.

### INTRODUCTION

The integrity of the gastro duodenal mucosa depends on the balance between aggressive (acid, pepsin, *Helicobacter pylori* infection, non-steroidal anti-inflammatory drugs-NSAID) and defensive (gastric mucus secretion, bicarbonate, blood flow and prostaglandins -PG-) factors. Gastric ulcers develop when this balance is lost, which leads to local injury due to active inflammation.[1-5]Increased oxidative stress due to the augmented generation of reactive oxygen species (ROS), one of the pathogenic events involved in the development of gastric ulceration, is mainly generated in the neutrophils. [6-10]

D-002, a mixture of six higher aliphatic primary alcohols (C<sub>24</sub>, C<sub>26</sub>, C<sub>28</sub>, C<sub>30</sub>, C<sub>32</sub>, C<sub>34</sub>) purified from beeswax, wherein triacontanol (C<sub>30</sub>) is the major component has been shown to protect against NSAID, ethanol, pylorus ligation, water restrain stress and acetic acid-induced ulcers, [11-16]and to alleviate the symptoms of subjects with duodenal ulcer or with gastritis symptoms.[17-20]D-002 has also shown to improve ulcer healing in patients with duodenal ulcers as well.[17]

The protective effects of D-002 against gastric mucosal damage involve multiple mechanisms, like increased secretion of gastric mucus, improved mucus composition, and reduction of lipid peroxidation, all demonstrated in the gastric mucosa of rats. [13-15]

The model of acetic acid-induced gastric ulcers in the rat is useful for investigating the efficacy of potential gastroprotective substances because the procedure is simple, ulcer sizes and severity are quite reproducible, and the pathological features and healing process mimic those of the human ulcers, including the spontaneous relapse and the good response to proton pump inhibitors (PPI), H2-receptor antagonists (H<sub>2</sub>A) and mucoprotective agents. [10]

A previous study demonstrated that D-002 administered orally at 200 mg/kg, not at 50 or 100 mg/kg, effectively healed the acetic acid-induced gastric ulcers in rats.[12] That experiment, however, neither compared the effects of D-002 with those of PPI or H2RA, nor assessed ulcer extents and re-epithelization histologically.

In light of these facts, this study compared the effects of D-002, omeprazole (a PPI), and ranitidine (a H2RA) on acetic acid-induced

gastric ulcers in the rat, and on the associated neutrophil infiltration and angiogenesis in the ulcerated areas.

### MATERIALS AND METHODS

#### Animals

Adult male Sprague Dawley rats (200-250g) were acquired in the National Centre for Laboratory Animal Production (CENPALAB, Havana) and adapted to the following laboratory conditions: temperature 22-23 °C, humidity 55-60%, and 12 hours dark/light cycles for 7 days, conditions that remained for the entire experiment. Free access to water and standard chow (rodent pellets from CENPALAB) was allowed.

The study was conducted in accordance with the Cuban Guidelines for the Laboratory Animals Care and Good Laboratory Practices. An independent ethic board for animal use approved the protocol for the study.

#### Administration and dosage

The batch of D-002, supplied by the Plants of Natural Products (National Centre for Scientific Research, Havana, Cuba), had the following composition, assessed with a validated gas chromatographic method:[21] tetracosanol (7.2%), hexacosanol (11.3%), octacosanol (13.9%), triacontanol (32.4%), dotriacontanol (22.9%) and tetratriacontanol (2.5%). Purity (total content of these alcohols) was 90.2%.

Ranitidine and omeprazole were acquired in the Cuban Pharmaceutical Industry (QUIMEFA) (Havana, Cuba). All treatments were prepared in acacia gum/water (1%) vehicle, 1 hour prior to the experiment.

Twenty-four hours after the ulcer induction the rats were randomized into eight groups of 10 rats each: a vehicle control and seven groups with acetic acid-induced ulceration: a vehicle control, two D-002 (200 and 400 mg/kg, respectively), two omeprazole (5 and 10 mg/kg), and two ranitidine (25 and 50 mg/kg) groups. Treatments were given by oral gastric gavage (1 mL/kg) for 5 consecutive days. At the end of the treatment, rats were fasted for 24 hours, then anesthetized under ether atmosphere and sacrificed.

### Acetic acid ulcer induction

Gastric ulcers were induced by applying locally acetic acid on the anterior serosal surface of the glandular stomach, as previously reported.[16] Briefly, 50  $\mu$ L of 80% acetic acid were applied to the serosal surface of glandular portion by using a round ring of 10 mm in diameter. Twenty seconds later, the acid solution was removed, wiped with filter paper and the abdomen was closed. Thereafter, rats fed normally and received orally the treatments (vehicle, D-002, omeprazole or ranitidine) for 5 days. The rats were then sacrificed and their stomachs were removed, opened along the greater curvature and the mucosal surface exposed, washed with normal saline, stretched and pinned on cork board. Two independent blinded observers examined stomachs under the light with a magnifying glass. Each lesion was measured along longest longitude. Five petechiae were considered equivalent to a 1 mm ulcer.[22]

### Histological analysis

Small pieces of stomach samples, including the ulcers, were fixed in phosphate-buffered formaldehyde, dehydrated, embedded in paraffin, and 5  $\mu$ m-thick sections were cut and stained with haematoxylin and eosin for light microscopic evaluation. Samples from all the rats were taken. Ulcers characteristics and healing, like regeneration of the ulcerated mucosa, formation of granulation tissue, glands arrangement and inflammatory infiltrate were evaluated. [23]

The infiltration of polymorphonuclear (PMN) neutrophils into the gastric mucosa was quantified by counting the cells in each cross-section of mucosa in accordance to Nygard et al (1994) [24] and Noa et al (1998). [25] The mean PMN counts were determined for three sections per animal in each group. The expressed mean was calculated from the mean value of each animal and afterwards averaged for each group.

Angiogenesis was determined when the formation of new microvessels was observed in the lamina propria or granulation tissue. A semi-quantitative assessment of the angiogenesis was performed with the following score: 0) none vessels per field, 1) a discrete amount of angiogenesis (1 or two vessels per field), 2) a moderate amount of angiogenesis (2 or three vessels per field), and 3) a great amount of angiogenesis (more than three vessels per field). Results were the mean  $\pm$  ES of eight rats per group.

### Statistical analyses

Ulcer index and neutrophil per ulcerated areas were presented as means  $\pm$  SEM and angiogenesis the mean  $\pm$  ES. Statistical comparisons were done by using the one-Way ANOVA test followed by Dunnett's multiple comparison tests or the Mann Whitney U Test, as corresponded. Statistical significance was set at  $p < 0.05$ . Data were processed with the Statistics Software for Windows (Release 4.2 Stat Soft Inc, Tulsa OK, US).

### RESULTS

Five days after serosal application of acetic acid, round and deep gastric ulcers were observed in all the positive control rats.

Repeat doses of all treatments significantly reduced acetic acid-induced ulcers (Table 1). D-002 (200 and 400 mg/kg) produced significant reductions of 49% ( $p < 0.01$ ) and 60% ( $p < 0.001$ ), respectively, omeprazole (5 and 10 mg/kg) decreased the ulcers by 39% ( $p < 0.05$ ) and 61% ( $p < 0.001$ ), respectively; and ranitidine (25 and 50 mg/kg) by 52% ( $p < 0.01$ ) and 68% ( $p < 0.001$ ), respectively, all compared to the control group. The reductions achieved with the highest doses of all treatments were greater than those achieved with the lowest doses and similar among groups.

**Table 1: Effects of D-002, omeprazole and ranitidine on acetic acid-induced ulcers**

| Treatment        | Doses (mg/kg) | Ulcer index (mm)   | Inhibition (%) |
|------------------|---------------|--------------------|----------------|
| Negative Control | -             | 0                  | -              |
| Positive Control | -             | 18.3 $\pm$ 1.98    | -              |
| D-002            | 200           | 9.35 $\pm$ 2.37**  | 49             |
| D-002            | 400           | 7.28 $\pm$ 1.18*** | 60             |
| Omeprazole       | 5             | 11.10 $\pm$ 1.79*  | 39             |
| Omeprazole       | 10            | 7.20 $\pm$ 2.00*** | 61             |
| Ranitidine       | 25            | 8.85 $\pm$ 1.18**  | 52             |
| Ranitidine       | 50            | 5.80 $\pm$ 1.81*** | 68             |

Values are expressed as mean  $\pm$  S.E.M for ten animals. Vehicle and all drugs were given orally, \*  $p < 0.05$ , \*\*\*  $p < 0.001$ . Comparisons with positive control (acetic acid + vehicle control). All comparisons were performed using one-way analysis of variance (Dunnett's test)

Positive control rats exhibited the characteristic histological pattern of acetic acid induced gastric ulcers, showing damaged mucosal epithelium, distortion of glands, severe inflammatory infiltrate, proliferation of fibroblasts and cellular debris in the ulcerated wall of stomach (Fig. 1a), while the negative control rats did not exhibit such changes (Fig. 1b). By contrast, rats of all treated groups showed healing signs, such as reductions of ulcer sizes and inflammatory infiltrates, with some extent of mucosal regeneration (re-epithelization), glandular organization, and proliferation of connective tissue cells (granulation tissue). Angiogenesis, observed

in all treated groups, was significantly increased in D-002 and omeprazole, not in ranitidine, treated groups ( $p < 0.001$  for both doses of D-002 and the highest of omeprazole,  $p < 0.01$  for the lowest dose of omeprazole), as compared to the positive controls. Ranitidine tended to increase angiogenesis, albeit not significantly, versus the controls ( $p = 0.058$ ) (Table 2 and Figs.1c-1h).

All treatments decreased significantly the number of PMN leukocytes per ulcerated area as compared to the positive control group, without significant differences among them. (Table 2)

**Table 2: Effect of D-002, omeprazole and ranitidine on acetic acid-induced neutrophil infiltration and angiogenesis in the gastric tissue**

| Treatments                 | Doses (mg/kg) | Neutrophil per ulcerated areas* | Angiogenesis score-            |
|----------------------------|---------------|---------------------------------|--------------------------------|
| Negative control           | -             | 80.8 $\pm$ 1.59 +++             | -                              |
| Positive control (vehicle) | -             | 150.9 $\pm$ 0.69                | 0.5 $\pm$ 0.18                 |
| D-002                      | 200           | 99.7 $\pm$ 0.86+++              | 2.25 $\pm$ 0.16+++             |
| D-002                      | 400           | 88.0 $\pm$ 0.63+++              | 3.0 $\pm$ 0.12+++              |
| Ranitidine                 | 25            | 92.7 $\pm$ 0.51+++              | 1.12 $\pm$ 0.12 <sup>†</sup>   |
| Ranitidine                 | 50            | 89.8 $\pm$ 0.63+++              | 1.12 $\pm$ 0.12 <sup>§§§</sup> |
| Omeprazole                 | 5             | 97.0 $\pm$ 0.93+++              | 1.75 $\pm$ 0.16++              |
| Omeprazole                 | 10            | 90.1 $\pm$ 1.03+++              | 2.00 $\pm$ 0.12+++             |

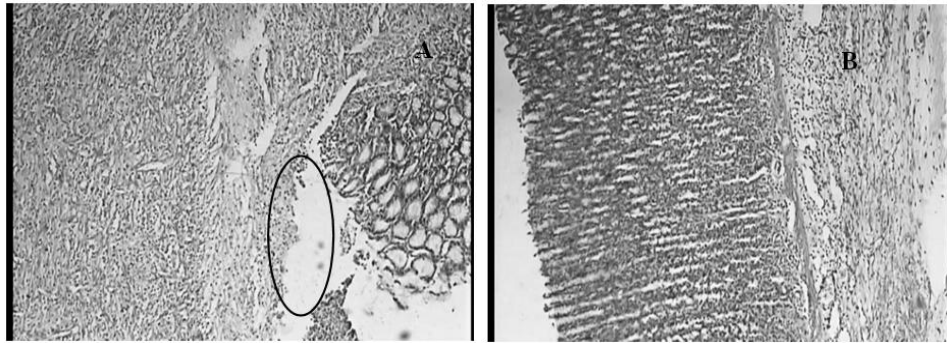
Data expressed as means  $\pm$  SME,

+  $p < 0.05$  ++  $p < 0.001$ , <sup>†</sup> $p = 0.058$ . Comparisons with positive control (Mann Whitney U test)

D-002 400 vs Omeprazole 10 <sup>§§§</sup>  $p < 0.001$ ; D-002 400 vs Ranitidine 50 <sup>§§§</sup>  $p < 0.001$  (Mann Whitney U test)

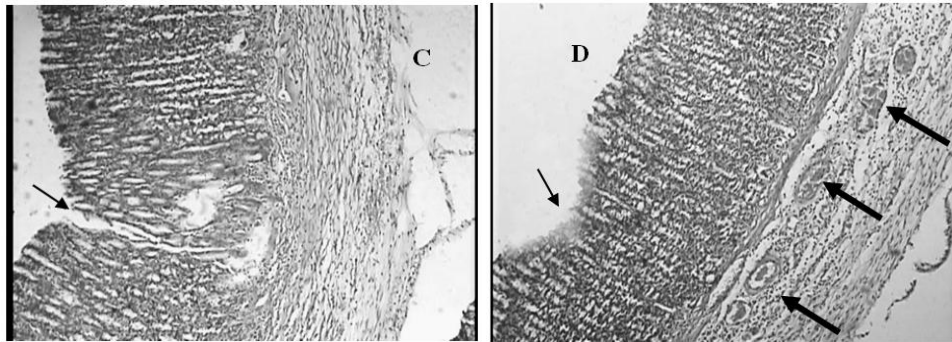
**Figures: 1a.** Severe gastric ulcer formation observed in a positive control rat (ellipsis). **1b.** No damage observed in a negative control rat. Examples of moderate gastric ulcerations observed in D-002 (200 and 400mg/kg) **1(c-d)**; ranitidine (25 and 50 mg/kg) **1(e-f)** and

omeprazole (5 and 10 mg/kg) **1(g-h)** treated rats. Healing signs such as reduced ulcer sizes (thin arrow), angiogenesis more marked in D-002 400 mg (width arrow), and decreased inflammatory infiltrate were observed in all treated groups. Haematoxylin and eosin 100X.

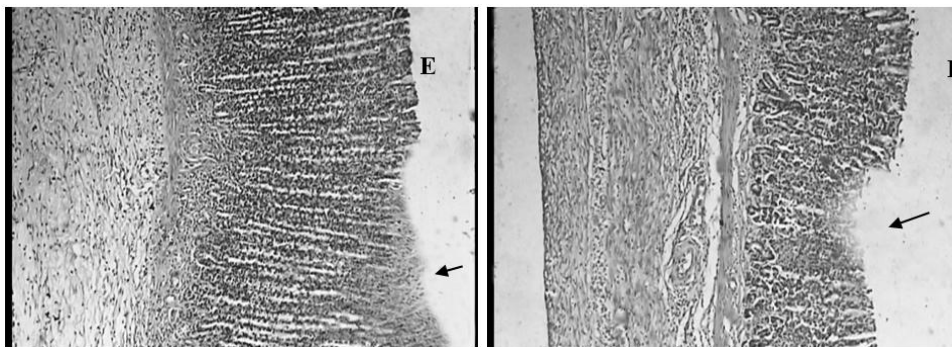


**Fig. 1a: Positive control Fig. 1b: Negative control**

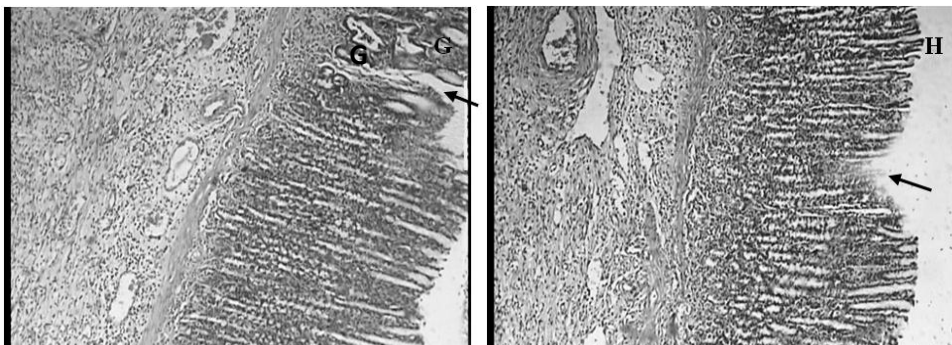
Stomachs of rats with acetic acid-induced ulcers and treated with:



**Fig. 1c: D-002 200 mg/kg Fig 1d: D-002 400 mg/kg**



**Fig. 1e: Ranitidine 25 mg/kg Fig 1f: Ranitidine 50 mg/kg**



**Fig. 1g: Omeprazole 5 mg/kg Fig 1h: Omeprazole 10 mg/kg**

**Fig. 1: Microphotograph of stomachs of rats with acetic acid-induced ulcer and negative control**

## DISCUSSION

This study demonstrates that the healing effect of the highest doses of D-002, omeprazole and ranitidine were comparable, so that D-002 400 mg/kg, omeprazole 10 mg/kg and ranitidine 50 mg/kg produced ulcer reductions of 60%, 61% and 68%, respectively, as compared to the control group. Nevertheless, since a dose-graded response for the doses tested was obtained for each treatment, and we did not evaluate higher doses, we ignore if the same occurred for higher doses.

The local application of acetic acid on the serosal surface of the glandular stomach produced characteristic gastric ulcers that were decreased by omeprazole and ranitidine, consistent with other data, [10][25-26] which confirms the validity of this model in our conditions, and then the results here described. The ulcer reduction (39%) with omeprazole 5 mg/kg given for 5 days here found is consistent with the ulcer decreases (39% and 70%, respectively) achieved with omeprazole (2 mg/kg/day) given for 5 and 15 days, [10,23,26] so that the healing effect increased with the treatment duration. Some authors, however, have reported conflicting results as oral omeprazole 10 mg/kg given for the same time failed to heal acetic acid gastric ulcers.[27,28]

The healing effects of D-002, omeprazole and ranitidine were confirmed by the histopathological study. The controls displayed the histological characteristics of acetic acid-induced gastric ulcers mentioned above, while rats treated exhibited an attenuated pattern. Histologically, the ulcerated areas of all treated groups exhibited granulation tissue consisting of connective tissue (collagen) and epithelium, growing extensively and supplying microvessels for restoration of the microvascular network and connective tissue cells for restoration of the lamina propria within the mucosal scar. Angiogenesis, the formation of new microvessels from pre-existing vessels, is a key factor for ulcer healing as it allows the supply of nutrients and oxygen to the damaged area.[29] Histological evidences of this process were seen in D-002 and omeprazole-treated rats, mainly in the rats treated with D-002 (400 mg/kg).

The healing of acetic acid-induced gastric ulcers in rats by enhancing angiogenesis should not be surprising, since a similar effect has been reported for an extract of *Cochinchina momordica* seeds (SK-MS10).[29]

Acetic acid-induced ulcers better resembles human gastric and duodenal ulcers in location, chronicity and severity, being accepted as the best model for studying the effects of treatments on the healing process. Chronic ulcers induced by acetic acid are mainly due to an increased volume of acid output, subsequent pyloric obstruction and mucosal necrosis. It is logical, therefore, that antisecretory substances, like PPI and H2RA accelerate the healing of these ulcers.[10,27] Nevertheless, non antisecretory mechanisms, like those elicited by antioxidant substances, are also implicated in the healing of these ulcers.

The occurrence of neutrophils infiltration was increased significantly in the positive control as compared to the negative control group, which suggest that oxygen derived free radicals derived from neutrophils may play a role in the development of acetic acid gastric ulcers, through the production of superoxide anions mediating lipid peroxidation [30], having an inhibitory effect on gastric ulcers healing in rats. All treatments significantly decreased neutrophils infiltration in a similar extent, which could represent a reduction of this source of ROS during the healing process. This appreciation, however, is merely speculative as this study did not assess any oxidative variable in the gastric mucosa. Nevertheless, other substances that have demonstrated to be effective on this model have rendered similar results, so that the healing-promoting effect of teprenone on acetic acid-induced gastric ulcers in rats seem to be due not only to stimulation of gastric mucus secretion, but also to the inhibition of neutrophil infiltration and lipid peroxidation in the ulcerated gastric tissue, [29] and several substances that promote ulcer healing, such as resveratrol, ginger and *Vaccinium myrtillus* L. extracts, act by decreasing the accumulation of neutrophils and the associated release of oxygen-derived free radicals. [30-33] Likewise, omeprazole, one of the

reference drugs used in the study, has been shown to exert gastroprotection by acting as antioxidant. [34]

The fact that D-002 is as effective as omeprazole and ranitidine for healing acetic acid-induced gastric ulcers suggests that its healing effect could be clinically relevant not only for reducing the symptoms, but also for healing the ulcers, [17-20] as occurred in patients with duodenal ulcers.[17] Previous studies demonstrated that D-002 is devoid of antisecretory effects, and that produce gastroprotective effects through increased gastric mucus secretion and/or antioxidant effects. Although the elucidation of the mechanism whereby D-002 accelerates the healing of acetic acid-induced ulcer was beyond the objectives of this work, we demonstrate in this study that inhibition of neutrophil infiltration and angiogenesis are processes associated to the healing effect of D-002 on acetic acid-induced ulcers in the rat.

In conclusion, this study demonstrates, by the first time, that the ability of D-002 for healing acetic acid-induced ulcers is comparable to that of OME and RAN. This effect was associated to the reduction of neutrophil infiltration and the increase of restorative angiogenesis into the ulcerated areas

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge the technical assistance of Ms. Nilda Mendoza of Pharmacology Department of Centre of Natural Product, National Centre of Scientific Research.

## REFERENCES

- Ramakrishnan K, Dinas RC. Peptic Ulcer Disease. Am Fam Physician.2007;76:1005-12.
- Lamarque D. Pathogenesis of gastroduodenal lesions induced by non-steroidal anti-inflammatory drugs. Gastroenterol Clin Biol 2004; 28:C18.
- Allen A and Flemström G. Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin. Am J Cell Physiol 2005; 288:C1-19.
- Laine L, Takeuchi K, Tarnawski A. Gastric mucosal defense and cytoprotection: bench to bedside. Gastroenterology 2008; 135: 41-60.
- Phillipson M, Johansson ME, Henriksnäs J, Petersson J, Gendler SJ, Sandler S, Persson AE, Hansson GC, Holm L. The gastric mucus layers: constituents and regulation of accumulation. Am J Physiol Gastrointest Liver Physiol 2008; 295:G806-12.
- Demir S, Yilmaz M, Koseoglu M, Akalin N, Aslan D, Aydin A. Role of free radicals in peptic ulcer and gastritis. Turkish J Gastroenterology 2003; 14: 39-43.
- Jainu M, Devi S. Attenuation of neutrophil infiltration and proinflammatory cytokines by *Cissus quadrangularis*: a possible prevention against gastric ulcerogenesis. J Herb Pharmacother 2005; 5:33-42.
- Naito Y, Takagi T, Katada K, Tomatsuri N, Mizushima K, Handa O, Kokura S, Yagi N, Ichikawa H, Yoshikawa T. Gastric peroxisome proliferator activator receptor- $\gamma$  expression and cytoprotective actions of its ligands against ischemia-reperfusion injury in rats. J Clin Biochem Nutr 2011; 48:170-77.
- Yasukawa K, Kasazaki K, Hyodo F, Utsumi H. Non-invasive analysis of reactive oxygen species generated in rats with water immersion restraint-induced gastric lesions using in vivo electron spin resonance spectroscopy. Free Radic Res 2004; 38:147-55.
- Okabe S, Amagase K. An overview of acetic acid ulcer models-the history and state of the art of peptic ulcer research. Biol Pharm Bul 2005; 28:1321-41.
- Más R: D-002: A product obtained from beeswax. Drugs of the Future. 2001; 26: 731-44.
- Carbajal D, Molina V, Valdés S, Arruzazabala ML, Más R. Anti-ulcer activity of higher primary alcohols of beeswax. J Pharm Pharmacol 1995; 47:731-33.
- Carbajal D, Molina V, Valdés S, Arruzazabala ML, Rodeiro I, Más R, Magraner J. Possible cytoprotective mechanism in rats of D-002 an anti-ulcerogenic product isolated from beeswax. J Pharm Pharmacol 1996; 48:858-60.

14. Carbajal D, Molina V, Noa M, Valdes S, Arruzazabala ML, Aguiar A, Más R. Effects of D-002 on gastric mucus composition in ethanol-induced ulcer. *Pharmacol Res* 2000; 42:329-32.
15. Molina V, Valdés S, Carbajal D, Arruzazabala ML, Menéndez R, Más R. Antioxidant effects of D-002 on gastric mucosa of rats with experimentally-induced injury. *J Med Food* 2001; 4:79-83.
16. Molina V, Carbajal D, Arruzazabala ML, Mas R. Therapeutic effect of D-002 (Abexol) on gastric ulcer induced experimentally in rats. *J Med Food*. 2005; 18:59-62.
17. Hano O, Illnait J, Mas R, Fernández L, Piñol F, Fernández J. Effects of D-002, a Product Isolated from Beeswax, on Duodenal Ulcer: A Double-Blind, Placebo-Controlled Study. *Curr Ther Res*. 2001; 62: 394-07.
18. Illnait J, Terry H, Más R, Fernandez L, Carbajal D. Effects of D-002, a product isolated from beeswax, on gastric symptoms of patients with osteoarthritis treated with piroxicam: a pilot study. *J Med Food*. 2005; 8: 63-8.
19. Fernández L, Terry H, Quiñones AM, Díaz B, Hernández ML, Illnait J, Más R. Effects of Abexol® in middle-aged and older subjects: an open follow-up. *Revista CENIC Ciencias Biológicas* 2008; 39: 3-8.
20. Rodríguez I, Illnait J, Terry H, Más R, Fernandez L, Fernández JC. Effects of Abexol® (beeswax alcohols) on gastrointestinal symptoms of middle-aged and older subjects assessed *Revista CENIC Ciencias Biológicas* 2009, 40: 147 -54.
21. González V, Marrero D, Sierra R, Velázquez C, Vicente R. Nuevo método por cromatografía gaseosa capilar para el análisis del ingrediente activo D-002. *Revista CENIC Ciencias Químicas* 2008; 39: 123-24.
22. Ohara A, Sugiyama S, Hoshino H, Hamajima E, Goto H, Tsukamoto Y, Ozawa T. Reduction of adverse effects of indomethacin by anti-allergic drugs in rat stomachs. *Arzneim-Forsch* 1992, 42:1115-8.
23. Dharmani, PK, Kumar V, Srivastava S, Palit G. Ulcer healing effect of anti-ulcer agents: a comparative study. *Internet J Acad Phys Assistants* 2003; 3: 2-10.
24. Nygard G, Anthony A, Piasecki C, Trevethick M, Hudson M, Dhilon A, Pounder RE, Wakefield AJ. Acute indomethacin-induced jejuna injury in the rat. Early morphological and biochemical changes. *Gastroenterology* 1994; 106: 567-75.
25. Noa M, Más R, Carbajal D, Valdés S. Effect of D-002 on acetic acid-induced colitis in rats at single and repeated doses. *Pharmacol Res* 2000; 41: 392-95.
26. Prasanth Reddy V, Sudheshna G, Afsar SK, Sai Saran S, Nelson Kumar S, Raja Ram C, Ravindra Reddy K. Evaluation of anti-ulcer activity of *Citrullus colocynthis* fruit against pylorus ligation induced ulcers in male Wistar rats. *Int J Pharm Pharm Sci* 2012;4: 446-51.
27. Amagase K. An overview of acetic acid ulcer models and their utility for drug screening. *Nippon Yakurigaku Zasshi* 2003; 122:73-92.
28. Prabha T, Dorababu M, Goel S, Agarwal PK, Singh A, Joshi VK, Goel RK. Effect of methanolic extract of *Pongamia pinnata* Linn seed on gastro-duodenal ulceration and mucosal defensive and defensive factors in rats. *Indian J Exper Biol* 2009; 47: 649-59.
29. Jung Mook Kang, Nayoung Kim, Bongcheol Kim, Joo-Hyon Kim, Bong-Yong Lee, Ji Hyun Park, Mi Kyoung Lee, Hye Seung Lee, Joo Sung Kim, Hyun Chae Jung. Enhancement of Gastric Ulcer Healing and Angiogenesis by *Cochinchina momordica* Seed Extract in Rats. *J Korean Med Sc* 2010; J25 (6): 875-81.
31. Kobayashi T, Ohta Y, Yoshino J, Nakazawa T. Teprenone promotes the healing of acetic acid-induced chronic gastric ulcers in rats by inhibiting neutrophil infiltration and lipid peroxidation in ulcerated gastric tissues. *Pharmacol Res* 2001; 43: 23-30.
32. Solmaz Ali, Sener Goksel, Cetinel Sule, Yuksel Meral, Yeğen Cumhur, Yeğen Berrak. Protective and therapeutic effects of resveratrol on acetic acid-induced gastric ulcer. *Free Radic Res* 2009; 43:594-603.
33. Ko JK, Leung CC. Ginger extract and polaprezinc exert gastroprotective actions by anti-oxidant and growth factor modulating effects in rats. *J Gastroenterol Hepatol* 2010; 25: 1861-69.
34. Ogawa K, Oyagi A, Tanaka J, Kobayashi S, Hara H. The Protective Effect and Action Mechanism of *Vaccinium myrtillos* L. on Gastric Ulcer in Mice. *Phytother Res* 2011, 25:1160-65.
35. Kaushik Biswas, Uday Bandyopadhyay, Ishita Chattopadhyay, Archana Varadaraj, Esahak Ali, Ranajit K Banerjee. A Novel Antioxidant and Antiapoptotic Role of Omeprazole to Block Gastric Ulcer through Scavenging of Hydroxyl Radical. *J Biol Chem* 2003; 278: 10993-11001.