PRODUCTO NATURAL

# Effects of policosanol on gastroprotective action of D-002

## Efectos del policosanol en la acción gastroprotectora del D-002

**Dra. Daisy Carbajal Quintana, Dra. Vivian Molina Cuevas, MSc. Yazmin Ravelo Calzado, Dra. Zullyt Zamora Rodríguez , Dr. Rosa Mas Ferreiro**

Centre of Natural Products. National Centre for Scientific Research. Havana, Cuba.

**ABSTRACT**

**Introduction:** policosanol, a mixture of higher aliphatic alcohols purified from sugar cane wax, is used to treat hypercholesterolemia. D-002 (Abexol), a mixture of higher aliphatic alcohols from beeswax, is an antioxidant supplement with gastroprotective effects. Then, concomitant intake of D-002 and policosanol may occur in routine practice, so potential pharmacological interactions between them should be researched on.
**Objective:** to find out the influence of policosanol on the gastroprotective effect of D-002 on the ethanol-induced gastric ulcer model.
**Methods:** rats were randomized into eight groups: one treated with the vehicle (control), two with D-002 (25 and 200 mg/kg), two with policosanol (25 and 200 mg/kg), two with the same doses of D-002 + policosanol and other with sucralfate (100 mg/kg). Treatments were given as single oral doses. One hour after treatment, rats received 60% ethanol orally and one hour later they were killed and their stomachs exposed. Effects on ulcer indexes (UI) were assessed.
**Results:** acute oral administration of D-002 (25 and 200 mg/kg) significantly reduced the ulcer indexes by 40 % and 68 %, respectively, as compared to the control group, and policosanol by 26 % and 47 %, respectively. The concomitant administration of the same doses of D-002 and policosanol significantly decreased ulcer indexes by 64 % (both given at 25 mg/kg) and by 92 % (both given at 200 mg/kg) as compared to the respective monotherapies. Sucralfate (100 mg/kg) significantly reduced (≅ 99 %) ulcer indexes compared to the control group.
**Conclusions:** the concomitant oral administration of policosanol with D-00 2 gives greater gastroprotection than D-002 monotherapy, so both products can be taken together.

**Keywords:** policosanol, bees wax alcohols, combined therapy, gastric ulcers, ethanol-induced ulcers.

**RESUMEN**

**Introducción:** el policosanol, mezcla de alcoholes alifáticos de alto peso molecular obtenida de la cera de caña de azúcar (*Saccharum officinarum* L), se emplea en el tratamiento de la hipercolesterolemia. El D-002 (Abexol), mezcla de alcoholes alifáticos obtenida de la cera de abejas, es un suplemento antioxidante con efectos gastroprotectores. Así, el consumo concomitante de D-002 y policosanol puede ocurrir en la práctica rutinaria, por lo cual algunas interacciones entre ellos deben ser investigadas.
**Objetivo:** determinar la influencia del policosanol sobre el efecto gastroprotector del D-002 en el modelo de úlcera gástrica inducida por etanol
**Métodos:** las ratas se distribuyeron en ocho grupos: uno tratado con el vehículo (control), dos con D-002 (25 y 200 mg/kg), dos con policosanol (25 y 200 mg/kg), dos con las mismas dosis de D-002 + policosanol, y otro con sucralfato (100 mg/kg). Los tratamientos se administraron como dosis únicas orales. Una hora después las ratas recibieron por vía oral etanol 60 % y se sacrificaron; los estómagos se extrajeron y se cuantificó el índice de úlceras.
**Resultados:** la administración oral aguda de D-002 (25 y 200 mg/kg) redujo significativamente el índice de úlceras en un 40 % y un 68 %, respectivamente, con respecto al grupo control y el policosanol en un 26 % y un 47 %, respectivamente. La administración concomitante de D-002 y policosanol redujo significativamente el índice de úlceras en un 64% (ambos administrados a 25 mg/kg) y un 92 % (ambos administrados a 250 mg/kg) al compararse con las respectivas monoterapias. Sucralfato (100 mg/kg) redujo significativa y marcadamente (≅ 99 %) el índice de úlceras con respecto al grupo control.
**Conclusiones:** la administración oral concomitante de policosanol más D-002 confiere una gastroprotección mayor que las respectivas monoterapias, de modo que pueden ser administrados conjuntamente.

**Palabras clave:** policosanol, alcoholes alifáticos de la cera de abejas, úlcera gástrica, etanol.

## INTRODUCTION

Peptic ulcer disease has been a major cause of morbidity and mortality worldwide, but the development of effective acid suppressants and treatment of *Helicobacter pylori* infection has contributed to lower its prevalence. However, this condition continues to be an important health issue, mainly in the elderly, because of the common use of non-steroidal anti-inflammatory drugs (NSAIDs) as well as due to other factors like smoking, alcohol intake, consumption of salted food and stress.[1-3] Indeed, NSAID-induced upper GI complications have been increasing as expanding elderly population, so that the proportion of NSAID ulcer tends to rise, due to the use of aspirin for vascular prevention and of other NSAIDs for rheumatologic/orthopaedic conditions, like osteoarthritis.[4,5] A similar trends occurs in Cuba.[6]

Ulcers that develop in the acid-peptic environment of the gastroduodenum, referred as peptic ulcers, do not develop spontaneously in a healthy mucosa, but it result from the unbalance between aggressive and defensive mucosa factors. The main aggressors are the content of acid and pepsin activity in gastric secretions, while protective factors include gastric mucus, mucosal microcirculation and motility,

cellular regeneration and endogenous protective agents, like prostaglandins (PG), nitric oxide and epidermal growth factors.[7-9]

The gastrotoxic effects of NSAIDs have been linked with the inhibition of cycloxygenase-1 (COX-1) and COX-2 and the concomitant reduction of PG synthesis, which turns to reduce gastric mucus thickness and mucosal blood flow.[10-12] Although the mechanisms whereby all aggressive cause mucosal damage are not identical, all include the increase of oxidative stress in the gastric mucosa.[13]

The pharmacological management of peptic ulcer diseases mainly includes the use of proton pump inhibitors and histamine 2-receptor antagonists, but antacids, mucoprotective drugs (sucralfate, prostaglandin analogs) and M1 muscarinic blockers are also used to treat these conditions. Although these drugs have shown to be effective, most produce adverse effects that may limit its use,[14-18] which makes rationale the search of effective and safer treatments.

D-002 (Abexol), a mixture of higher aliphatic alcohols from beeswax, is an antioxidant supplement with gastroprotective effects demonstrated in experimental[19-24] and clinical studies[25-27] associated with the reinforcement of defensive factors, like the increase in mucus secretion,[21] the improvement on mucus quality,[22] and the reduction of the generation of hydroxyl radical, lipid peroxidation and protein oxidation in the gastric mucosa.[23,24] In turn, policosanol a mixture of higher aliphatic alcohols purified from sugar cane wax (*Saccharum officinarum* L*)*, is a drug indicated to treat hypercholesterolemia.[28-31]

Taking into account the frequent occurrence of gastrointestinal complaints, even in apparently healthy individuals, and the frequency of hypercholesterolemia among middle-aged and older people in Cuba, the concomitant intake of D-002 and policosanol may occur in routine practice and some interactions between them should be investigated. In light of these issues, this study investigated whether policosanol can modify the efficacy of D-002 on ethanol-induced ulcers.

## METHODS

### ANIMALS

Male Sprague Dawley rats (250-300 g) from the National Centre for laboratory Animal Production (Cenpalab, Havana, Cuba) were adapted for 7 days to experimental conditions: controlled temperature (22-23 ºC, humidity 55-60 % and 12 h dark/light cycles. Water and food (rodent pellets from Cenpalab) were provided *ad libitum*, but the rats were deprived of food for 16 h prior to the experiment.

Study was conducted in accordance with the Cuban guidelines for the care of laboratory animals and Code of Good Laboratory Practice (GLP). An independent ethic committee for animal use approved the protocol for these experiments.

### ADMINISTRATION AND DOSAGE

The compositions of D-002 and policosanol batches, supplied by the Plants of Natural Products (Havana, Cuba) were assessed with validated gas chromatographic methods.[32,33] D-002 and policosanol were suspended in Tween

20/water (2 %) vehicle, and sucralfate (QUIMEFA, Cuba) in gum Arabic suspension (1 %). All treatments were administered orally by gastric gavage (1 mL/200 g).

Rats were randomized into eight groups of 10 rats each: a control group (vehicle) and seven groups administered with ethanol: 2 treated with D-002 (25 and 200 mg/kg), 2 with policosanol (25 and 200 mg/kg), 2 with D-002 + policosanol, and 1 with sucralfate (100 mg/kg). All animals receive only one administration. The doses used were based in previous experiments.

### INDUCTION OF GASTRIC ULCERS BY ETHANOL

One hour after oral dosing of vehicle, D-002, policosanol or sucralfate rats received orally ethanol (60 %), (1 mL/200 g). One hour later they were sacrificed under ether atmosphere for assessing the gastric ulceration.[34]

### EVALUATION OF GASTRIC MUCOSAL DAMAGE

After sacrifice, rat stomachs were immediately removed, opened along the greater curvature, and lesions were examined macroscopically. The ulcer lengths were assessed as the total sum of the lengths [mm] of the gastric lesions. Two independent blinded observers performed the observations and measurements of the lesions.[35]

### STATISTICAL ANALYSIS

Results were expressed as mean ± SEM (standard error of mean).Comparisons among groups were conducted with the nonparametric Kruskal Wallis test, while paired comparisons between treated and control groups were performed with the Mann-Whitney U test. Statistical significance was chosen for $\alpha = 0.05$. Data were processed with the Statistics Software for Windows (Release 4.2 Stat Soft Inc, Tulsa OK, US).

## RESULTS

Single oral doses of D-002 (25 and 200 mg/kg) significantly reduced ethanol-induced UI by 40 % and 68 %, respectively, as compared to the control group (table). In turn, policosanol (25 and 200 mg/kg) significantly decreased the ethanol-induced UI by 26 % and 47 %, respectively.

The concomitant administration of the same doses of D-002 and policosanol significantly decreased UI by 64 % (both given at 25 mg/kg) and 92 % (both given at 200 mg/kg) as compared to the respective monotherapies. Sucralfate (100 mg/kg), the reference drug, significantly and very markedly (≅ 99 %) reduced UI versus the control group.

**Table.** Effect of policosanol, D002 and policosanol + D-002 in ethanol-induced ulcer model

| Treatment | Dose (mg/kg) | Ulcer Index Mean ± SEM | Inhibition (%) |
|---|---|---|---|
| Control | | 69.83 ± 13.68 | - |
| D-002 | 25 | 41.90 ± 3.50* | 40 |
| D-002 | 200 | 22.08 ± 5.55**▽ | 68 |
| Policosanol | 25 | 51.30 ± 1.73 | 26 |
| Policosanol | 200 | 36.70 ± 3.39 * +++ | 47 |
| Policosanol + D-002 | 25 | 25.21 ± 4.89** | 64 |
| Policosanol + D-002 | 200 | 5.30 ± 2.07*** | 92 |
| Sucralfate | 100 | 0.62 ± 0.33*** | 99 |

Comparisons between groups were performed with the Mann-Whitney U test.
*p<0.05; ** p< 0.01; ***p< 0.001 comparison *vs.* control. ▽▽p< 0.01, ▽▽▽p< 0.001 comparison *vs.* policosanol + D-002 (25 mg). ++p< 0.01, +++p< 0.001 comparison *vs.* policosanol + D-002 (200 mg).

## DISCUSSION

The present study demonstrates that oral administration of policosanol (25 and 200 mg/kg) did not interfere with the gastroprotective effect of D-002 (25 and 200 mg/kg) when both substances were administered together. By the contrary, the concomitant treatment with both substances increased the protective effect of D-002 (64 % decrease of UI with 25 mg/kg, 92 % with 250 mg/kg) with respect to the reduction produced by monotherapies with D-002 25 and 250 mg/kg (40 % and 68 %, respectively) or policosanol (26 % and 47 %, respectively).

Ethanol-induced ulcers have been associated with gastric mucus depletion, increased free radical production and impairment of gastric microcirculation, all of which contributes to produce erosions, haemorrhage and necrosis on the gastric mucosa.[36]

The protective effect of D-002 (25 and 200 mg/kg) on ethanol-induced gastric ulcers here seen, which agrees with previous results,[20,23] due to multiple mechanisms, such as the increase of gastric mucus secretion, the improvement of mucus composition,[21,22] and antioxidant effects exerted on the gastric mucosa.[37,38] On its side, earlier studies had demonstrated that policosanol was also effective for protecting ethanol-induced ulcers,[39] but less effectively than D-002. The mechanism whereby policosanol produced such effect was not investigated. It is plausible to suppose that the antioxidant effect of policosanol[40] could contribute to this action, but such appreciation is merely speculative since no study has investigated whether policosanol exhibits such effect on the gastric mucosa.

The fact that both substances exhibit protective effects on ethanol-induced ulcers raised the possibility of an interaction between them on this model, making it a useful tool for investigating whether policosanol could interfere or improve the antiulcer effect of D-002. Accordingly, we found that the concomitant administration of policosanol improved, not reduced, the efficacy of D-002 on this model in an additive rather than in a synergic fashion.

Further studies should investigate the mechanisms underlying this interaction, which were beyond the objectives of this study. Our results, however, support that the concomitant administration of D-002 and policosanol should not ameliorate the gastroprotective effect of the former.

## CONCLUSIONS

The concomitant oral administration of policosanol with D-002 confers a higher gastroprotection than D-002 monotherapy, so that both products can be taken together.

## REFERENCES

1. Zapata-Colindres JC, Zepeda-Gómez S, Montaño-Loza A, Vázquez-Ballesteros E, Villalobos JJ, Valdovinos-Andraca F. The association of *Helicobacter pylori* infection and non-steroidal anti-inflammatory drugs in peptic ulcer disease. Can J Gastroenterol. 2006;20:277-80.

2. Sung JY, Tosí KF, Ma TK, Yung MY, Lau YW, Chiu WY. Causes of mortality in patients with peptic ulcer bleeding: a prospective cohort study of 10 428 cases. Am J Gastroenterol. 2010;105:84-9.

3. Zullo A, Hassan C, Campo SM, Morini S. Bleeding peptic ulcer in the elderly: risk factors and prevention strategies. Drugs Aging. 2007;24:815-28.

4. Shiotani A, Manabe N, Kamada T, Fujimura Y, Sakakibara T, Haruma K. Risk and preventive factors of low-dose aspirin-induced gastroduodenal injuries: a comprehensive review. Gastroenterol Hepatol. 2012;27(Suppl 3):8-12.

5. Iwamoto J, Saito Y, Honda A, Matsuzaki Y. Clinical features of gastroduodenal injury associated with long-term low-dose aspirin therapy. World J Gastroenterol. 2013;19:1673-82.

6. Ministerio de Salud Pública, Dirección Nacional de Registros Médicos y Estadísticas de Salud. Anuario Estadístico de Salud. La Habana: MINSAP; 2012.

7. Allen A, Flemström G. Gastroduodenal mucus bicarbonate barrier: Protection against acid and pepsin. Am J Physiol Cell Physiol. 2005;288:C1-C19.

8. Phillipson M, Johansson ME, Henriksnäs J, Petersson J, Gendler SJ, Sandler S, et al. The gastric mucus layers: constituents and regulation of accumulation. Am J Physiol Gastrointest Liver Physiol. 2008;295:G806-12.

9. Palileo C, Kaunitz JD. Gastrointestinal defense mechanisms. Curr Opin Gastroenterol. 2011;27:543-8.

10. Lamarque D. Pathogenesis of gastroduodenal lesions induced by non-steroidal anti-inflammatory drugs. Gastroenterol Clin Biol. 2004;28:C18-26.

11. Yoshikawa T, Naito Y. Pathogenesis of NSAIDs-induced gastrointestinal ulcers. Nihon Rinsho. 2011;69:995-1002.

12. Takeuchi K. Pathogenesis of NSAID-induced gastric damage: importance of cyclooxygenase inhibition and gastric hypermotility. World J Gastroenterol. 2012;18:2147-60.

13. Muresan A, Suciu S, Mitrea DR, Alb C, Login C, Crisan D, Daicoviciu D. Oxidative stress implications in experimental gastric ulcer induced by indomethacin. Bulletin UASVM, Veterinary Medicine. 2008;65(1):119-25.

14. Parikh N, Howden CW. The safety of drugs used in acid-related disorders and functional gastrointestinal disorders. Gastroenterol Clin North Am. 2010;39:529-42.

15. Fujikawa Y, Watanabe T, Tominaga K, Fujiwara Y, Sato H, Arakawa T. Efficacy of prostaglandin derivatives and mucoprotective drugs in treatment and prevention for NSAIDs-induced ulcer. Nihon Rinsho. 2011;69:1039-43.

16. Heidelbaugh JJ, Metz DC, Yang YX. Proton pump inhibitors: are they over utilised in clinical practice and do they pose significant risk? Int J Clin Pract. 2012;66:582-91.

17. Desilets AR, Asal NJ, Dunican KC. Considerations for the use of proton-pump inhibitors in older adults. Consult Pharm. 2012;27:114-10.

18. Roulet L, Vernaz N, Giostra E, Gasche Y, Desmeules J. Adverse effects of proton pump inhibitors: Should we worry about long-term exposure? Rev Med Interne. 2012;33(8):439-45.

19. Mas R. D-002: A product obtained from beeswax. Drugs of the Future. 2001;26(8):731-44.

20. Carbajal D, Molina V, Valdés S, Arruzazabala ML, Más R. Anti-ulcer activity of higher primary alcohols of beeswax. J Pharm Pharmacol. 1995;47:731-3.

21. Carbajal D, Molina V, Valdés S, Arruzazabala ML, Rodeiro I, Más R, et al. Possible cytoprotective mechanism in rats of D-002 an anti-ulcerogenic product isolated from beeswax. J Pharm Pharmacol. 1996;(48):858-60.

22. Carbajal D, Molina V, Noa M, Valdes S, Arruzazabala ML, Aguiar A, et al. Effects of D-002 on gastric mucus composition in ethanol-induced ulcer. Pharmacol Res. 2000;42(4):329-32.

23. Molina V, Valdés S, Carbajal D, Arruzazabala ML, Menéndez R, Mas R. Antioxidant effects of D-002 on gastric mucosa of rats with experimentally-induced injury. J Med Food. 2001;4:79-83.

24. Pérez Y, Oyárzabal A, Mas R, Molina V, Jiménez S. Protective effect of D-002, a mixture of beeswax alcohols, against indomethacin-induced gastric ulcers and mechanism of action. Int J Nat Med. 2013;67(1):182-9.

25. 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;62:394-407.

26. Illnait J, Terry H, Mas 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(1):63-8.

27. Illnait J, Rodríguez I, Molina V, Mendoza S, Mas R, Fernández L, et al. Effects of D-002 (beeswax alcohols) on gastrointestinal symptoms and oxidative markers in middle-aged and older subjects. Lat Am J Pharm. 2013;32:166-74.

28. Castaño G, Fernández L, Illnait J, Fernández J, Mas R, Mesa M, et al. Effects of policosanol on patients with borderline to mildly increased serum cholesterol levels: A prospective, double-blind placebo-controlled study. Curr Ther Res Clin & Exp. 2003;142(7):277-84.

29. Menéndez R, Más R, Amor AM, González RM, Fernández JC, Rodeiro I, et al. Effects of policosanol treatment on the susceptibility of low density lipoprotein (LDL) isolated from healthy volunteers to oxidative modification in vitro. Br J Clin Pharmacol. 2000;50:255-62.

30. Menéndez R, Más R, Amor A, Fernández JC, González RM. Effects of policosanol on the low density lipoprotein (LDL) isolated on hypercholesterolemic patients at high coronary risk to in vitro copper-medíated lipid peroxidation. Curr Ther Res. 2000;61:609-20.

31. Noa M, Mas R. Effect of policosanol on atherosclerotic plaque composition on aortas of *Macaca arctoides* monkeys. Arch Med Res. 2005;36:441-7.

32. González V, Marrero D, Sierra R, Velázquez C, Vicente R. Nuevo método por cromatografía gaseosa capilar para el análisis del ingrediente activo D-002. Rev CENIC Ciencias Químicas. 2008;9:123-5.

33. Marrero D, González V, Sierra R, Velázquez C. Validación de un nuevo método analítico por CG con columna capilar para la determinación de alcoholes alifáticos de alto peso molecular en el policosanol ingrediente activo. Rev Colombiana de Ciencias Quim Farm. 2008;37(1):62-8.

34. Zengil H, Onuk E, Ercan ZS, Turker RK. Protective effect of Iloprost and UK 38 485 against gastric mucosal damage induced by various stimuli. Prostagl Leukotr Med. 1987;30:61-7.

35. Ohara A, Sugiyama S, Hoshino H, Hamajima E, Goto H, Tsukamoto Y. Reduction of adverse effects of indomethacin by anti-allergic drugs in rat stomachs. Arzneim-Forsch Drug Res. 1992;42:1115-8.

36. Muhammed Ashraf VK, Thamotharan G, Sengottuvelu S, Haja Sherief S, Sivakumar T. Evaluation of anti ulcer activity of *Ficus pumila* L. Leaf extract in albino rats. GJRMI. 2012;1(8):340-51.

37. Pérez Y, Oyarzábal A, Ravelo Y, Mas R, Jiménez S, Molina V. Inhibition of COX and 5-LOX enzymes by D-002 (beeswax alcohols). Curr Top Nutr Res (Forthcoming). 2012.

38. Pérez Y, Oyarzábal A, Jiménez S, Molina V, Mas R. Efecto secuestrador del D-002 sobre radicales hidroxilo en mucosa gástrica. Rev Cubana Farm. 2012;46(1):87-96.

39. Arruzazabala ML, Carbajal D, Molina V, Valdés S, Más R, García M. Estudio farmacológico de la interacción entre el policosanol y la aspirina en animales de experimentación. Rev Iberoam Tromb Hemost. 1992;5:17-20.

40. Oyarzábal A, Molina V, Jiménez S, Curveco D, Mas R. Efectos del policosanol, el extracto de semillas de uva y su terapia combinada sobre marcadores oxidativos en ratas. Rev Cubana Farm. 2010;45(1):87-96.

Recibido: 20 de junio de 2014.
Aprobado: 28 de julio de 2014.

*Daisy Carbajal Quintana.* Centre of Natural Products. 199 street e/19 & 21. Atabey, Playa, Havana, Cuba. Email: cpn.sup@cnic.edu.cu