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# One-year Dog Toxicity Study of D-002, a Mixture of Aliphatic Alcohols

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Key words: D-002; aliphatic primary alcohols; anti-ulcer drugs; beagle dogs; chronic toxicity.

**D-002 is a mixture of high-molecular-weight aliphatic alcohols, obtained from bees wax (*Apis mellifera*), with mild anti-inflammatory properties and effective anti-ulcer activities demonstrated in experimental models. This study investigated the oral toxicity of D-002 administered for 1 year to beagle dogs. Twenty-four beagle dogs (12 males and 12 females) were distributed randomly in three experimental groups (four animals per group): a control and two treated groups received D-002 at 50 and 250 mg $kg^{-1}$ (7 days/week) by gastric gavage. Overall, D-002 was well tolerated throughout the study. No signs or symptoms of toxicity were observed, and no mortality occurred during the study. All groups showed similar weight gain and food consumption. No hematological, blood biochemical or histopathological disturbances attributable to treatment were observed. This study shows no drug-related toxicity induced by long-term administration of up to 250 mg $kg^{-1}$ D-002 to beagle dogs. Copyright © 2001 John Wiley & Sons, Ltd.**

## INTRODUCTION

D-002 is a natural mixture of high-molecular-weight alcohols, isolated and purified from bees wax, containing triacontanol, octacosanol, dotriacontanol, hexacosanol, tetracosanol and tetratriacontanol as a minor component. Oral administration of D-002 induces a mild anti-inflammatory effect. It reduces the weight of the cotton-pellet granuloma and reduces leukotriene $B_4$ ($LTB_4$) levels in the exudate of carragenan-induced pleurisy.[1]

Nevertheless, no gastric mucosa damage was induced by its oral administration up to doses of 1000 mg $kg^{-1}$ and its action is considered to differ from that of non-steroidal anti-inflammatory drugs. The basis of its anti-inflammatory activity is related to the reduction of $LTB_4$ levels. Leukotrienes play a role in the etiology of gastric mucosal damage.[2]

On the other hand, remarkable anti-ulcer effects of D-002 have also been demonstrated in different experimental models. Thus, D-002 at 5–50 mg $kg^{-1}$ prevents ulcers induced by ethanol (60%) and HCL (0.6 M). At 30 mg $kg^{-1}$, D-002 also prevents indomethacin-induced ulcers and inhibits ulcers induced in pylorus-ligated rats. These doses did not affect the volume and pH of acid secretion. These effects seem to be mediated by a reinforcement of the defensive mechanism of the gastric mucosa.[3,4]

The acute oral $LD_{50}$ of D-002 in rats, mice, rabbits and dogs is 5 g $kg^{-1}$.[5] Repeated-dose studies for 3 months and 1 year in rats revealed no drug-related toxicity at doses up to 650 mg $kg^{-1}$day and 1 g $kg^{-1}$ $day^{-1}$, respectively.[6] In addition, studies on D002 genotoxicity and mutagenicity, as well as those undertaken to evaluate its effects on fertility and reproduction, have revealed no drug-related toxicity.[7,8]

Taking into account that anti-ulcer drugs must be administered repeatedly because of the recurrence of the disease, the aim of this work was to investigate the toxicity of D-002 administered orally for 1 year to beagle dogs.

## MATERIALS AND METHODS

### Animals

Twenty-four young adult beagle dogs (10–12 weeks) of both genders were obtained from Centro Nacional de Animales de Laboratorio (CENPALAB, Cuba), weighing 8–12 kg at the start of the study. Animals were adapted to experimental conditions for 15 days prior to treatment, the temperature was 25 ± 2°C and the humidity was 50–60%. Daily clinical observations were made to control their health status. Only animals considered to be clinically healthy were included in the study. For this purpose, the clinical examination included gross observations of the overall condition of the animal, such as behaviour, respiratory or gastrointestinal diseases and any congenital anomalies. In addition, the examination was performed for the detection of ectoparasites. Animals were housed in individual cubicles, provided with dog-chow obtained from CENPALAB and received water *ad libitum*.[9,10]

### Administration and dosage

D-002 was administered as a suspension in a vehicle containing 10 mg acacia gum $ml^{-1}$ $H_2O$. The composition

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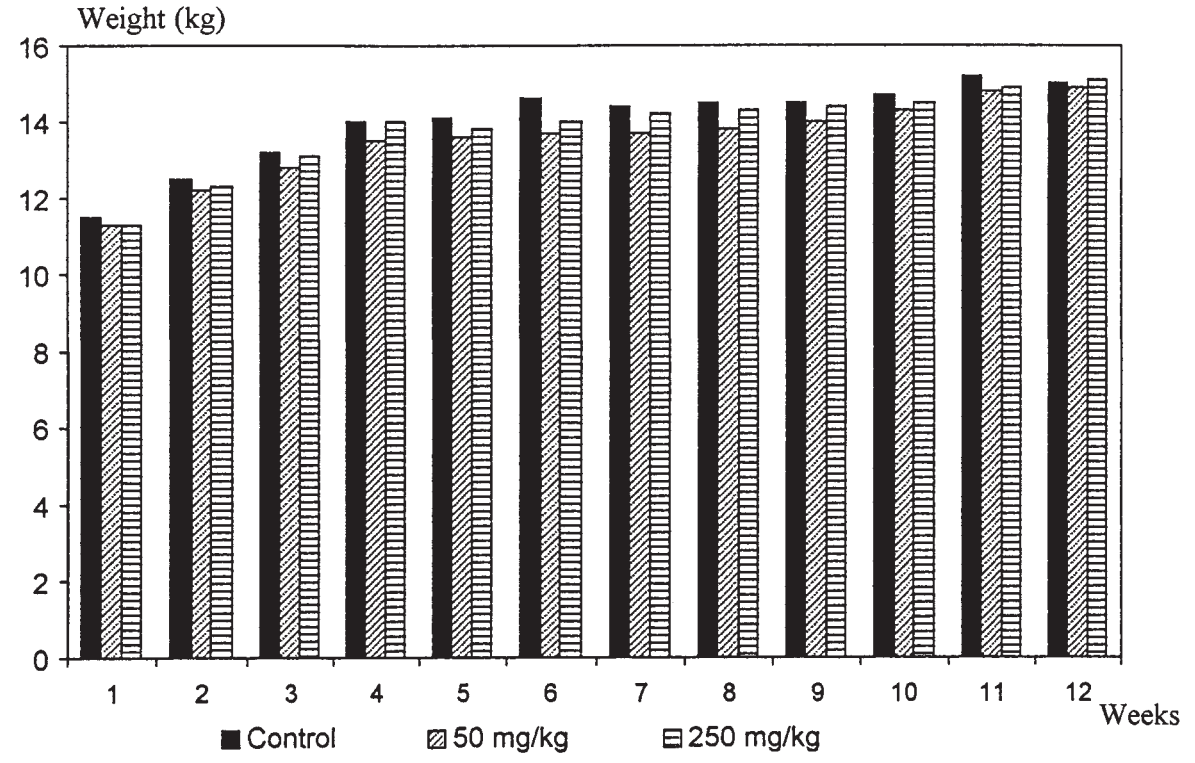


**Figure 1.** Body weight gain of male dogs orally administered D-002 for 1 year.

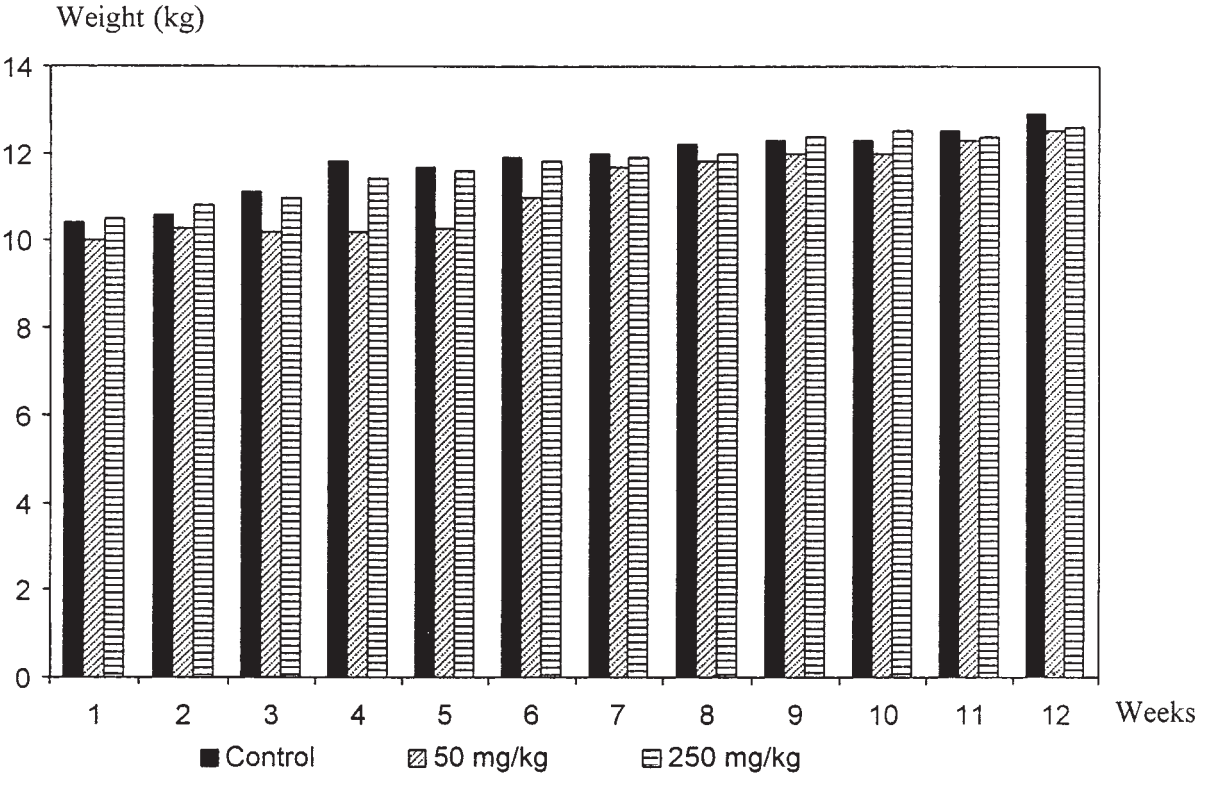


**Figure 2.** Body weight gain of female dogs orally administered D-002 for 1 year.

of the batch used in this study was as follows: triacontanol, 26.63%; octacosanol, 17.49%; dotriacontanol, 16.95%; hexacosanol, 15.34%; tetracosanol, 13.24% and tetratriacontanol, 2.23%. The batch used had a 92% purity, according to the quality criteria specifications checked by gas chromatography. The impurities of D-002 consist of diols, hydrocarbons or potassium salts of fatty acids, all of which are present in beewax.

The stability of the test material was studied previously in our Chemistry department, indicating that

 

**Table 1. Biochemical and hematological parameters of male beagle dogs orally treated with D-002 for 1 year (mean ± standard deviation)[a]**

| Group | $t = 0$ | $t = 1$ | $t = 2$ | $t = 3$ | $t = 4$ |
|---|---|---|---|---|---|
| Glucose (mmol $l^{-1}$) | | | | | |
| Control | 4.18 ± 0.24 | 4.16 ± 0.18 | 4.17 ± 0.13 | 4.09 ± 0.04 | 3.87 ± 0.33 |
| 50 mg $kg^{-1}$ | 4.20 ± 0.30 | 4.13 ± 0.20 | 4.16 ± 0.17 | 4.19 ± 0.32 | 4.00 ± 0.11 |
| 250 mg $kg^{-1}$ | 4.18 ± 0.24 | 4.14 ± 0.25 | 4.19 ± 0.19 | 4.06 ± 0.43 | 3.84 ± 0.24 |
| Alanine transferase (IU) | | | | | |
| Control | 32.77 ± 2.95 | 34.99 ± 11.16 | 39.95 ± 7.93 | 31.15 ± 7.72 | 29.58 ± 5.92 |
| 50 mg $kg^{-1}$ | 28.50 ± 2.81 | 31.24 ± 7.65 | 36.87 ± 6.30 | 32.40 ± 4.70 | 27.73 ± 7.82 |
| 250 mg $kg^{-1}$ | 29.42 ± 3.53 | 29.31 ± 5.60 | 39.35 ± 6.12 | 33.78 ± 3.84 | 28.85 ± 7.58 |
| Aspartate transferase (IU) | | | | | |
| Control | 21.00 ± 4.96 | 20.00 ± 3.16 | 27.00 ± 7.53 | 21.50 ± 5.97 | 22.25 ± 6.07 |
| 50 mg $kg^{-1}$ | 21.75 ± 2.75 | 17.75 ± 1.71 | 25.25 ± 8.42 | 25.25 ± 6.18 | 24.75 ± 6.95 |
| 250 mg $kg^{-1}$ | 18.75 ± 1.50 | 17.25 ± 3.30 | 25.75 ± 4.79 | 22.75 ± 3.86 | 22.75 ± 5.25 |
| Acid phosphatase (IU) | | | | | |
| Control | 5.06 ± 0.65 | 4.76 ± 0.77 | 4.11 ± 0.68 | 3.73 ± 0.61 | 3.09 ± 0.34 |
| 50 mg $kg^{-1}$ | 4.89 ± 0.63 | 4.29 ± 0.56 | 4.31 ± 0.68 | 3.65 ± 0.57 | 3.07 ± 0.36 |
| 250 mg $kg^{-1}$ | 5.40 ± 0.71 | 4.36 ± 1.25 | 4.42 ± 0.67 | 3.58 ± 0.83 | 3.04 ± 0.32 |
| Alkaline phosphatase (IU) | | | | | |
| Control | 52.5 ± 13.3 | 53.0 ± 13.5 | 55.0 ± 11.4 | 44.8 ± 8.5 | 41.5 ± 9.3 |
| 50 mg $kg^{-1}$ | 64.0 ± 13.0 | 62.3 ± 11.5 | 58.2 ± 9.3 | 44.0 ± 4.3 | 38.3 ± 6.3 |
| 250 mg $kg^{-1}$ | 57.3 ± 16.6 | 58.3 ± 15.1 | 57.0 ± 16.4 | 43.5 ± 5.8 | 35.5 ± 3.9 |
| Creatinine (mmol $l^{-1}$) | | | | | |
| Control | 75.2 ± 7.61 | 75.30 ± 3.91 | 75.72 ± 4.96 | 73.80 ± 3.48 | 71.02 ± 2.56 |
| 50 mg $kg^{-1}$ | 72.9 ± 7.76 | 78.45 ± 2.02 | 77.18 ± 3.61 | 73.20 ± 2.95 | 70.90 ± 3.23 |
| 250 mg $kg^{-1}$ | 73.9 ± 5.08 | 74.98 ± 3.81 | 75.40 ± 5.49 | 71.28 ± 4.63 | 69.63 ± 4.84 |
| Acetylcholinesterase (U) | | | | | |
| Control | 0.51 ± 0.06 | 0.53 ± 0.07 | 0.57 ± 0.09 | 0.43 ± 0.04 | 0.41 ± 0.08 |
| 50 mg $kg^{-1}$ | 0.52 ± 0.06 | 0.52 ± 0.07 | 0.52 ± 0.08 | 0.42 ± 0.05 | 0.35 ± 0.12 |
| 250 mg $kg^{-1}$ | 0.51 ± 0.05 | 0.53 ± 0.07 | 0.53 ± 0.06 | 0.43 ± 0.04 | 0.37 ± 0.18 |
| Hemoglobin (g 100 $ml^{-1}$) | | | | | |
| Control | 14.08 ± 0.90 | 14.53 ± 0.40 | 14.73 ± 0.49 | 14.60 ± 0.88 | 14.73 ± 0.71 |
| 50 mg $kg^{-1}$ | 14.20 ± 0.42 | 14.08 ± 0.83 | 14.23 ± 0.66 | 14.68 ± 0.90 | 14.55 ± 0.34 |
| 250 mg $kg^{-1}$ | 14.33 ± 0.84 | 14.70 ± 0.47 | 14.28 ± 0.43 | 14.00 ± 0.45 | 14.53 ± 0.43 |
| Hematocrit (%) | | | | | |
| Control | 43.75 ± 1.89 | 43.25 ± 2.5 | 44.50 ± 2.39 | 45.75 ± 3.50 | 46.75 ± 2.06 |
| 50 mg $kg^{-1}$ | 43.25 ± 2.75 | 44.50 ± 4.5 | 46.75 ± 3.30 | 46.25 ± 5.12 | 46.50 ± 2.39 |
| 250 mg $kg^{-1}$ | 44.75 ± 0.96 | 46.00 ± 1.4 | 44.75 ± 3.40 | 44.50 ± 1.91 | 46.75 ± 2.99 |

[a] $t_0$ = baseline; $t_1$, $t_2$, $t_3$ = three-month intervals; $t_4$ = the end of 1 year.

D-002 is stable under our laboratory conditions for time periods over 1 year. Also, the homogeneity of the suspensions used during the assays was checked periodically by gas chromatographic methods.

Suspensions was made weekly according to body weight gain. Administration was done daily by gastric gavage (8 ml $kg^{-1}$) for 1 year. Dogs were randomly assigned to three groups (four males and four females per group): a control group received only the vehicle and two others were treated with D-002 at 50 and 250 mg $kg^{-1}$ daily doses, respectively.

The rationale for the dose selection was based on the pharmacological endpoints. D-002 (5–25 mg $kg^{-1}$) was effective in an experimental model of indomethacin-induced ulcers and also in preventing ethanol-induced ulcers. The same doses administered to normal rats significantly increased the soluble mucus content, prevented the increase of vascular permeability induced by ethanol (60%) and reduced the concentration of thromboxane $B_2$ in gastric mucosa of rats with ethanol-induced ulcers.[1,3,4] The highest level was 250 mg $kg^{-1}$ $day^{-1}$; taking into account the solubility properties of D-002, suspensions with concentrations of >250 mg $kg^{-1}$ $day^{-1}$ required more than once-a-day gastric gavage manipulation and consequently were not recommended for long-term studies.

## Observations

Animals were observed daily from 9 to 12 a.m. during the whole study, including a complete physical examination. General activity, food intake, respiration, urine

**Table 2. Biochemical and hematological parameters of female beagle dogs orally treated with D-002 for 1 year (mean ± standard deviation)[a]**

| Group | $t = 0$ | $t = 1$ | $t = 2$ | $t = 3$ | $t = 4$ |
|---|---|---|---|---|---|
| Glucose (mmol $l^{-1}$) | | | | | |
| Control | 4.18 ± 0.17 | 4.17 ± 0.22 | 4.35 ± 0.19 | 4.23 ± 0.65 | 4.12 ± 0.45 |
| 50 mg $kg^{-1}$ | 4.18 ± 0.17 | 4.16 ± 0.15 | 4.35 ± 0.17 | 4.33 ± 0.37 | 4.00 ± 0.43 |
| 250 mg $kg^{-1}$ | 4.15 ± 0.18 | 4.17 ± 0.18 | 4.35 ± 0.19 | 4.22 ± 0.71 | 4.25 ± 0.43 |
| Alanine transferase (IU) | | | | | |
| Control | 32.9 ± 6.42 | 33.2 ± 8.71 | 36.1 ± 6.80 | 28.8 ± 5.79 | 28.6 ± 5.27 |
| 50 mg $kg^{-1}$ | 30.6 ± 8.92 | 36.8 ± 8.41 | 36.5 ± 4.92 | 28.4 ± 4.56 | 31.0 ± 11.18 |
| 250 mg $kg^{-1}$ | 31.4 ± 9.54 | 31.4 ± 8.21 | 34.0 ± 7.36 | 27.9 ± 9.23 | 28.0 ± 5.91 |
| Aspartate transferase (IU) | | | | | |
| Control | 22.00 ± 3.92 | 16.50 ± 2.65 | 28.25 ± 8.06 | 21.50 ± 3.00 | 23.75 ± 3.95 |
| 50 mg $kg^{-1}$ | 22.75 ± 9.71 | 16.00 ± 2.45 | 23.50 ± 10.41 | 22.50 ± 7.90 | 21.75 ± 5.85 |
| 250 mg $kg^{-1}$ | 18.75 ± 5.74 | 14.75 ± 3.77 | 23.25 ± 7.41 | 21.75 ± 4.92 | 20.75 ± 2.22 |
| Acid phosphatase (IU) | | | | | |
| Control | 5.17 ± 1.08 | 4.06 ± 0.54 | 4.01 ± 0.19 | 3.53 ± 0.59 | 3.43 ± 0.66 |
| 50 mg $kg^{-1}$ | 5.23 ± 0.83 | 3.81 ± 0.57 | 3.93 ± 0.28 | 3.56 ± 0.66 | 3.30 ± 0.57 |
| 250 mg $kg^{-1}$ | 4.84 ± 1.28 | 3.82 ± 0.78 | 3.76 ± 0.75 | 3.43 ± 0.79 | 3.25 ± 0.80 |
| Alkaline phosphatase (IU) | | | | | |
| Control | 53.5 ± 15.0 | 57.5 ± 13.4 | 49.0 ± 10.9 | 44.5 ± 12.2 | 36.0 ± 5.0 |
| 50 mg $kg^{-1}$ | 61.5 ± 9.1 | 60.3 ± 11.1 | 60.2 ± 3.2 | 54.3 ± 7.5 | 42.0 ± 12.4 |
| 250 mg $kg^{-1}$ | 60.8 ± 11.2 | 55.5 ± 9.7 | 49.0 ± 14.9 | 43.5 ± 8.8 | 33.0 ± 6.1 |
| Creatinine (mmol $l^{-1}$) | | | | | |
| Control | 77.88 ± 8.20 | 74.92 ± 5.09 | 76.55 ± 3.59 | 72.65 ± 8.48 | 68.63 ± 6.58 |
| 50 mg $kg^{-1}$ | 74.53 ± 4.30 | 70.25 ± 9.62 | 70.80 ± 4.22 | 71.50 ± 4.67 | 69.70 ± 5.53 |
| 250 mg $kg^{-1}$ | 75.03 ± 8.26 | 76.28 ± 11.95 | 77.13 ± 10.32 | 76.78 ± 11.51 | 70.95 ± 13.19 |
| Acetylcholinesterase (U) | | | | | |
| Control | 0.44 ± 0.11 | 0.47 ± 0.11 | 0.45 ± 0.17 | 0.33 ± 0.06 | 0.37 ± 0.09 |
| 50 mg $kg^{-1}$ | 0.50 ± 0.11 | 0.49 ± 0.12 | 0.51 ± 0.13 | 0.37 ± 0.06 | 0.38 ± 0.10 |
| 250 mg $kg^{-1}$ | 0.45 ± 0.12 | 0.44 ± 0.11 | 0.48 ± 0.15 | 0.32 ± 0.06 | 0.33 ± 0.11 |
| Hemoglobin (g 100 $ml^{-1}$) | | | | | |
| Control | 14.48 ± 0.81 | 14.78 ± 1.74 | 14.55 ± 0.53 | 14.70 ± 0.54 | 14.60 ± 0.43 |
| 50 mg $kg^{-1}$ | 15.08 ± 0.86 | 14.95 ± 0.74 | 14.70 ± 0.65 | 14.70 ± 0.88 | 14.63 ± 0.74 |
| 250 mg $kg^{-1}$ | 14.38 ± 0.63 | 14.43 ± 0.78 | 14.50 ± 0.54 | 14.63 ± 0.95 | 14.68 ± 0.73 |
| Hematoint (%) | | | | | |
| Control | 44.25 ± 4.92 | 43.25 ± 5.44 | 44.50 ± 2.52 | 45.75 ± 2.22 | 45.50 ± 2.65 |
| 50 mg $kg^{-1}$ | 45.00 ± 3.37 | 47.00 ± 2.94 | 46.00 ± 2.94 | 42.00 ± 3.65 | 43.25 ± 2.63 |
| 250 mg $kg^{-1}$ | 44.75 ± 3.77 | 45.00 ± 3.27 | 45.50 ± 2.65 | 42.50 ± 3.70 | 44.00 ± 3.92 |

[a] $t_0$ = baseline; $t_1$, $t_2$, $t_3$ = three-month intervals; $t_4$ = the end, of 1 year.

and feces quality, skin and mucosa aspects were recorded. Body weights were recorded monthly.

Blood samples were drawn from the jugular vein for hematological and blood biochemistry determinations at baseline (the day before starting the administrations) and every 3 months up to the end of the study. The following determinations were made on the serum samples: glucose, aspartate and alanine transferases, acid and alkaline phosphatases and creatinine. All determinations of blood biochemistry were made by enzymatic methods, using reagent kits from Boehringer Mannhein (Germany). In addition, acetyl cholinesterase, hemoglobin and hematocrit were measured on blood samples.

## Pathological study

After 1 year of therapy, animals were sacrificed. They were anesthetized with sodium pentobarbital (30 mg $kg^{-1}$). When the parpebral reflex was absent and muscular flaccidity appeared, animals were exsanguinated through the jugular veins. Animals were randomly sacrificed and histological analysis was performed 'blindly'. Abdominal, thoracic and cranial cavities were examined *in situ* and eviscerated. Organ weights were determined and organ weight/body weight ratio × 100 was calculated for the statistical analysis. Samples from brain, cerebellum, medula, hypophysis, eyes, larynx, trachea, bronchii, lungs, tongue, esophagus, salivary glands, stomach, duodenum,

**Table 3. Organ weights of male beagle dogs orally treated with D-002 for 1 year (mean ± standard deviation)**

| Group | Liver | Kidneys | | Heart | Lungs | Spleen |
|---|---|---|---|---|---|---|
| | | Right | Left | | | |
| Control | 2.35 ± 0.37 | 0.21 ± 0.03 | 0.21 ± 0.02 | 0.70 ± 0.03 | 0.65 ± 0.08 | 0.16 ± 0.02 |
| 50 mg $kg^{-1}$ | 2.85 ± 0.79 | 0.25 ± 0.06 | 0.25 ± 0.08 | 0.77 ± 0.10 | 0.69 ± 0.17 | 0.19 ± 0.01 |
| 250 mg $kg^{-1}$ | 2.80 ± 0.55 | 0.23 ± 0.06 | 0.24 ± 0.06 | 0.76 ± 0.05 | 0.64 ± 0.10 | 0.18 ± 0.01 |
| | Thymus | Adrenals | | Testis | | Prostate |
| | | Right | Left | Right | Left | |
| Control | 0.06 ± 0.01 | 0.002 ± 0.005 | 0.005 ± 0.005 | 0.09 ± 0.006 | 0.08 ± 0.006 | 0.06 ± 0.01 |
| 50 mg $kg^{-1}$ | 0.07 ± 0.02 | 0.005 ± 0.005 | 0.005 ± 0.005 | 0.09 ± 0.010 | 0.09 ± 0.010 | 0.06 ± 0.01 |
| 250 mg $kg^{-1}$ | 0.07 ± 0.01 | 0.002 ± 0.005 | 0.005 ± 0.005 | 0.08 ± 0.005 | 0.08 ± 0.005 | 0.06 ± 0.01 |

**Table 4. Organ weights of female beagle dogs orally treated with D-002 for 1 year (mean ± standard deviation)**

| Group | Liver | Kidneys | | Heart | Lung | Spleen |
|---|---|---|---|---|---|---|
| | | Right | Left | | | |
| Control | 2.41 ± 0.15 | 0.20 ± 0.005 | 0.21 ± 0.02 | 0.72 ± 0.06 | 0.66 ± 0.11 | 0.17 ± 0.02 |
| 50 mg $kg^{-1}$ | 2.58 − 0.12 | 0.21 ± 0.003 | 0.21 ± 0.03 | 0.75 ± 0.10 | 0.61 ± 0.03 | 0.18 ± 0.03 |
| 250 mg $kg^{-1}$ | 2.64 ± 0.37 | 0.21 ± 0.003 | 0.21 ± 0.04 | 0.78 ± 0.08 | 0.79 ± 0.16 | 0.17 ± 0.02 |
| | Thymus | Adrenals | | | | |
| | | Right | Left | | | |
| Control | 0.07 ± 0.01 | 0.005 ± 0.006 | 0.005 ± 0.006 | | | |
| 50 mg $kg^{-1}$ | 0.09 ± 0.02 | 0.010 ± 0.001 | 0.010 ± 0.001 | | | |
| 250 mg $kg^{-1}$ | 0.07 ± 0.01 | 0.010 ± 0.005 | 0.008 ± 0.005 | | | |

jejunum, ileum, cecum, colon, rectus, thymus, pancreas, liver, skeletal muscle, aorta, heart, bone marrow, skin, kidneys, adrenals, bladder, tests, penis, prostate, uterus, vagina, ovaries, lymph node, thyroid, parathyroid and bone were taken.[10] For microscopic analysis, tissues were fixed in buffered 10% formaldehyde and embedded in paraffin; sections were stained with hematoxylin and eosin. An Olympus BH2 microscope was used for the observations.

### Statistical analysis

Body weight, organ weight and blood parameters were analyzed through a variance analysis (Kruskall–Wallis non-parametric ANOVA test). Histopathological results were compared using Fisher's exact test. Analysis were done using the css/pc statistical package program and $P < 0.05$ was established for statistical significance.[11]

## RESULTS AND DISCUSSION

### Mortality analysis, clinical examinations and body weight data

No deaths occurred during the study, and chronic treatment with D-002 was well tolerated by animals. On the other hand, no clinical symptoms of toxicity were observed. Figures 1 and 2 show the body weight gain during the study. As can be observed, no significant differences between groups were found.

### Effects of D-002 on blood safety indicators

No significant differences were detected in any comparison of the hematological and blood biochemical parameters (Table 1 and 2).

### Anatomo-pathological study

At the autopsy, the appearance of organs and cavities was normal in all animals. Analysis of organ weight data showed no significant differences between treated and control groups for eithergender (Tables 3 and 4).

The microscopic study showed that a female beagle dog of the control group presented autoimmune thyroiditis, with the presence of a lymphocytic infiltrate with plasma cells forming aggregates in the interstitium, between the thyroid follicles. These lymphoplasmacytic infiltrates in beagle dog thyroid are associated with autoimmune-related thyroid damage.[12] Two male dogs treated with D-002 (50–250 mg $kg^{-1}$ $day^{-1}$) show this type of lesion too, along with a non-specific

chronic adenitis Nevertheless, it cannot be discounted that this type of lesion may be related to an autoimmune reaction, because these histological changes are not easily distinguished in either case.[13] The microscopic study shows that D-002 did not induce any pathological lesions attributable to treatment. Comparison between groups did not show significant differences (Table 5).

Because the stomach represents a target organ of anti-ulcer drug toxicity,[14,15] special attention was focused on this organ during the histopathological studies. Nevertheless, no damage on the gastric mucosa attributable to D-002 was observed, which is not surprising taking into account the cytoprotective mechanism postulated for the anti-ulcer effects of D-002.[4] The present results demonstrated that D-002 produced no untoward effects when administered for 1 year to beagle dogs up to a top oral dose of 250 mg $kg^{-1}$ $day^{-1}$. Because no drug-related toxicity was detected in this study, the highest dose evaluated could be considered a no-observable-effect level (NOEL).

**Table 5. Histopathological findings after 1 year of D-002 treatment**

| Observation | Group (mg $kg^{-1}$) | | |
|---|---|---|---|
| | Control | 50 | 250 |
| Males | | | |
| Adenitis | 0/4 | 1/4 | 1/4 |
| Thyroiditis | 0/4 | 1/4 | 1/4 |
| Females | | | |
| Thyroiditis | 1/4 | 0/4 | 0/4 |

## REFERENCES


1. Carbajal D, Molinal V, Valdés S, Arruzazabala L, Más R, Magraner J. Anti-inflammatory activity of D-002: an active product isolated from bees wax. *Prostagland. Leukotriene. Essent. Fatty Acids* 1998; **59**: 235–238.
2. Guslandi M. Gastric effects of leukotrienes. *Prostagland. Leukotriene. Med.* 1987; **26**: 203–208.
3. Carbajal D, Molina V, Valdés S, Arruzazabala L, Mas R. Effect of D-002: an active product isolated from beeswax on experimentally induced ulcers. *J. Pharm. Pharmacol.* 1995; **47**: 731–733.
4. Carbajal D, Molina V, Valdés S, Arruzazabala L, Rodeiro I, Mas R, Magraner L. Possible mechanism cytoprotective of D-002. *J. Pharm. Pharmacol.* 1996; **48**: 858–860.
5. Rodeiro I, Alemán C, Más R, Noa M, Briñis F, Hernandez C. Toxicología aguda del D-002 en ratas Sprague Dawley. *Rev. CENIC Cien. Biol.* 1995; **26**: 34–36.
6. Rodeiro I, Alemán C, Noa M, Menéndez R, Más R, Hernández C, García M. Pre-clinical oral toxicology in rats of D-002, a natural drug with anti-ulcer effects. *Drug Chem. Toxicol.* 1998; **21**: 151–162.
7. Rodeiro I, Gámez R, Acosta P, Fernández I, Más R, Alemán C. Estudio genotóxico del D-002, un producto con actividad antiulcerosa. *Rev. Españ. Toxicol.* 1998; **15**: 117–121.
8. Rodríguez MD, Gámez R, Rodríguez M, García H. Teratological evaluation of D-002 in rats and rabbits. *J. Appl. Toxicol.* 1998; **18**: 313–316.
9. Stevens KR, Mylecraine L. Issues in chronic toxicology. In *Principles and Methods of Toxicology* (3rd edn), Wallace Hayes A (ed.). Raven Press: New York, 1994.
10. Chhabra RS, Huff JE, Schwetz BS, Selkir KJ. An overview of pre-chronic and chronic toxicity/carcinogenicity experimental study designs and criteria used by the National Toxicology Program. *Environ. Health Perspect.* 1990; **86**: 313–312.
11. Gad S, Carrol S. Statistic for toxicologists. In *Principles and Methods of Toxicology* (2nd edn), Wallace Hayes A (ed.). Raven Press: New York, 1989.
12. Robbins S. *Patología Estructural y Funcional. Edición revolucionaria* (2nd edn). La Habana: Cuba, 1975.
13. Johannansen U, Kardevan A, Miroslav Z. *Lechrbuch der zpeziellen veterinar patologie* Vebgustav Fisher Verlag: Jena, 1986.
14. Ekman L, Hansson E, Havu N, Carlsson E, Lundberg C. Toxicological studies on omeprazole. Scand. J. Gastroenterol. 1985; **20**: 53–69.
15. Brimblecombe RW, Leslie GB, Walker TF, Toxicology of cimetidine. *Hum. Toxicol.* 1985; **4**: 13–25.


 