Journal of Food Protection, Vol. 71, No. 4, 2008, Pages 790–795

Acute Oral Safety Study of Rosemary Extracts in Rats

ARTURO ANADÓN,[1]* MARIA R. MARTÍNEZ-LARRAÑAGA,[1] MARIA A. MARTÍNEZ,[1] IRMA ARES,[1] MONICA R. GARCÍA-RISCO,[2] FRANCISCO J. SEÑORÁNS,[2] AND GUILLERMO REGLERO[2]

[1]*Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Universidad Complutense de Madrid, E-28040 Madrid, Spain; and* [2]*Sección Departamental Ciencias de la Alimentación, Universidad Autónoma de Madrid, Campus de Cantoblanco, E-28049 Madrid, Spain*

MS 07-352: Received 6 July 2007/Accepted 2 December 2007

ABSTRACT

Increasing interest in rosemary plants is due to their antioxidant and health-enhancing properties. The aim of this study was to evaluate the potential acute toxicity of two supercritical fluid extracts of rosemary. An acute safety study of rosemary extracts was conducted in Wistar rats at a single oral gavage dosage of 2,000 mg/kg of body weight. Rosemary extracts were well tolerated; no adverse effects or mortality were observed during the 2-week observation period. No abnormal signs, behavioral changes, body weight changes, or change in food and water consumption occurred. Two weeks after a single oral rosemary extract dose of 2,000 mg/kg of body weight, there were no changes in hematological and serum chemistry values, organ weights, or gross or histological characteristics. Rosemary extracts appear to have low acute toxicity, and the oral lethal doses (LD_{50}) for male and female rats are greater than 2,000 mg/kg of body weight.

Rosmarinus officinalis (rosemary) is an evergreen plant of the Lamiaceae (Labiatae) family, which also includes other plants such as *Thymus vulgaris* (thyme), *Mentha piperita* (mint), *Origanum vulgare* (oregano), *Lavandula officinalis* (lavender), *Hyssopus officinalis* (hyssop), *Ocimum basilicum* (basil), *Origanum majoran* (marjoram), and *Salvia officinalis* (sage). All these plants have been used for imparting desirable flavors to food and, in some cases such as rosemary, to prevent rancidity because of their inherent antioxidant activity *(2, 13, 26).* In folk medicine, rosemary has been used as a choleretic and a diuretic agent. Its volatile oil has been used in high-quality cosmetics, insect repellents, and remedies to treat minor gastrointestinal disorders, to stimulate the skin, and as an adjuvant in rheumatic and circulatory disorders *(16).* The European Scientific Cooperative on Phytotherapy *(9)* has recommended the internal use of rosemary leaf to improve hepatic and biliary function and to treat dyspeptic complaints and has recommended its external use as adjuvant therapy for rheumatic conditions, peripheral circulatory disorders, and promotion of wound healing and as a mild antiseptic. In traditional European medicine, rosemary has been used internally as a tonic, stimulant, and carminative to treat flatulence dyspepsia, stomach pains, headaches, and nervous tension. *(5).*

The aqueous extract of rosemary is reported to possess antihepatotoxic activity *(12, 15).* The antioxidant activity of some plant products may be parallel to their hepatoprotective (chemopreventive) and antimutagenic activities *(17, 25).* Studies have revealed that rosemary extracts inhibit phase I enzyme CYP450 activities and induce the expression of the phase II enzyme glutathione *S*-transferase *(18).*

Rosemary leaf contains phenolic acids (2 to 3% rosmarinic, chlorogenic, and caffeic acids), phenolic diterpenoid bitter substances (up to 4.6% carnosic acid, carnosol, rosmaridiphenol, and rosmanol), triterpenoid acids (oleanolic and ursolic acids), flavonoids (apigenin, luteolin, nepetin, and nepitrin), 1.2 to 2.5% volatile oils (15 to 50% 1,8-cineole, 15 to 25% α-pinene, 12 to 24% α-terpineol, 10 to 25% camphor, 5 to 10% camphene, 1 to 6% borneol, 1 to 5% bornyl acetate), and tannins *(3, 6, 9).* Although phenolic diterpenes, carnosic acid, carnosol, rosmanol, and epi- and iso-rosmanol are antioxidant compounds in rosemary leaves *(10, 17, 20, 22),* about 90% of the antioxidant activity of rosemary can be attributed to carnosol and carnosic acid *(1).* Supercritical fluid extraction has been suggested as a method for selective isolation of antioxidants from rosemary *(23)* mainly because of the mild processing conditions and selectivity *(21, 24).*

There is limited information about the toxicological safety of rosemary. Spurred by the growing interest in the potential use of rosemary leaf extracts as a dietary supplement and to establish toxicological safety data of these extracts, this research was undertaken to test the acute (limit dose) toxicity of two extracts of rosemary leaf in rats in an experimental design following the principles for the safety assessment of food additives *(27).*

MATERIALS AND METHODS

Test compound, reagents, chemicals, and materials. The *R. officinalis* L. sample consisted of recently collected dried rosemary leaves obtained from Herboristeria Murciana (Murcia, Spain). Antioxidant activity depends primarily on genetic and growing conditions (such as the quality of the original plant, its geographical origin, and climatic conditions), the harvesting date, and its storage and processing and secondarily on the extraction process and its selected parameters. The activity is related to the concentration of phenolic diterpenes in the rosemary extracts. To study the toxicity of representative rosemary extracts, two different samples of wild rosemary (A and B) harvested at different

* Author for correspondence. Tel: 34 91 3943834; Fax: 34 91 3943840; E-mail: anadon@vet.ucm.es.

times (autumn and spring 2005, respectively) and containing different concentrations of phenolic diterpenes, representing medium and high values found in commercial supercritical extracts, were tested. Rosemary leaves were dried using a traditional method previously described *(14).* Cryogenic grinding of the sample was performed under carbon dioxide, and particle size was determined by sieving the ground plant material to the appropriate size (between 999 and 500 μm). The whole sample with a moisture content of 7% was stored for a maximum of 4 months at −20°C until used to prevent changes in the antioxidant properties. 2,2-Diphenil-1-pycril hydrazyl hydrate (DPPH, 95% purity) was purchased from Sigma-Aldrich (Madrid, Spain), and carnosic acid (95%) and carnosol (96%) were obtained from Alexis Biochemical (Lausen, Switzerland). High-performance liquid chromatography (HPLC) grade methanol and acetonitrile were obtained from Lab Scan (Dublin, Ireland), acetic acid (99%) was from Merck Schuchardt (Hohenbrunn, Germany), Milli-Q water was from a purification system (Millipore, Billerica, Mass.), and CO_2 (N-48 quality) was from Air Liquide España S.A. (Madrid, Spain). Commercial corn oil (ASUA, Koipe, SOS Cuétara S.A., Madrid, Spain) was used as a vehicle to dissolve the rosemary extracts. All other chemicals were of the highest quality grade and were obtained from commercial sources.

Supercritical fluid extraction of rosemary. Extraction of the two different rosemary samples was carried out in a pilot-scale supercritical fluid extractor (Iberfluid, Barcelona, Spain) with a 285-ml extraction cell as previously described *(23).* Fractionation was achieved in two different separators assembled in series, with independent control of temperature and pressure, by either a decrease in pressure or a decrease in both pressure and temperature.

The extraction cell was filled with 60 g of ground rosemary and 60 g of washed sea sand (Panreac, Barcelona, Spain). Dynamic extraction was performed at 150 bar and 40°C (ρ_{ext} = 0.731 g/ml), and the extract was fractionated in two separators at 40°C, where pressures were set, in the first stage at 50% of extraction pressure (ρ_{ext} = 0.240 g/ml) and in the second stage at a fixed value of 20 bar (ρ_{ext} = 0.041 g/ml). Ethanol (7%) was used as a modifier. The addition of ethanol started after the selected pressure had been reached at half of the extraction time (60 min). The extracts were kept under N_2 at −20°C in the dark, and ethanol was eliminated at 35°C in a vacuum rotary evaporator.

Antioxidant activity assay. The antioxidant activity was determined by the DPPH scavenging assay based on a procedure described by Brand-Williams et al. *(4).* This method involved neutralization of free radicals of DPPH by the antioxidant extracts. For each sample, different concentrations were tested (from 1 to 10 μg/ml in the DPPH-methanol solution). The DPPH solution (1,950 μl of 23.5 μg/liter DPPH in methanol) was placed in test tubes, and 50 μl of the different concentrations of samples were added. Reactions were complete after 3 h at room temperature, and absorbance was measured at 516 nm in a UV-120-01 spectrophotometer (Shimazdu, Kyoto, Japan). Methanol was used to adjust the apparatus to zero, and the DPPH-methanol solution was used as a reference sample. The DPPH concentration in the reaction medium was calculated from the following calibration curve as determined by linear regression (r = 0.999): $y = 0.0247x - 0.0029$.

The percentage of remaining DPPH versus the extract concentration was then plotted to obtain the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% (EC_{50}). Thus, the lower the EC_{50}, the higher the antioxidant power. Each determination was repeated twice.

HPLC–diode array detection analysis. The analysis of the A and B extract samples was carried out in an HPLC apparatus (Varian Pro-star, Palo Alto, Calif.) equipped with a Nova Pack C_{18} column (Waters, Barcelona, Spain) of 15 by 4.6 mm and a 3.5-μm particle size. The mobile phase consisted of 1% acetic acid in acetonitrile (solvent A) and 1% acetic acid in water (solvent B) applied with the following gradient: 0 to 5 min, 50% B; 5 to 15 min, 50 to 30% B; 15 to 40 min, 30 to 0% B. The flow rate was constant at 0.7 ml/min. Injection volume was 20 μl, and detection was accomplished with a diode array detection system (Varian) monitoring the signal at a wavelength of 230 nm.

Acute oral toxicity test: animals and diets. Wistar male and female rats (Charles River Inc., Margate, Kent, UK) approximately 8 weeks of age were individually housed in polycarbonate cages with sawdust bedding and maintained in environmentally controlled rooms (22 ± 2°C and 50% ± 10% relative humidity) with a 12-h light-dark cycle (light from 8:00 a.m. to 8:00 p.m.). Food (A03 rodent diet, Scientific Animal Food and Engineering, Villemoisson-sur-Orge, France) and water were available ad libitum. The rats were divided into three groups of 12 animals each (6 males and 6 females). Group 1 (control) received corn oil orally, and groups 2 and 3 received extract A or B orally. This study was approved by the Ethics Committee of the Complutense University.

Dose preparation and administration. After an overnight fast, rats were weighed (162 to 181 g), and oral doses of A and B rosemary extracts were administered by gavage at a single limit dosage of 2,000 mg/kg of body weight. This dosage was selected on the basis of a preliminary study performed with single animals of one sex in which no evidence of toxicity was observed at oral dosages of 50 and 500 mg/kg of body weight. The extracts were prepared in an organic vehicle (corn oil), which contained 200 mg/ml extract A or extract B. The extracts were weighed, transferred to a foil-wrapped labeled container with a portion of the vehicle that had been heated to approximately 50°C, and mixed on a magnetic stir plate with a stir bar until the extract dissolved. The 200 mg/ml extract A or extract B preparation was prepared immediately prior to administration (10 ml/kg of body weight or 2 ml per rat weighing 200 g). Rats were observed for mortality and clinical signs. At the end of a 2-week observation period, the rats were examined by necropsy, and the weights of the organs were recorded.

Observations. The animals were observed twice daily (a.m. and p.m.) for mortality and morbidity. Detailed physical examinations of each animal were made prior to the study and daily until day 15. Rats were observed for their general condition and the condition of the skin and fur, eyes, nose, oral cavity, abdomen, and external genitalia, evaluated for respiration rate, and palpated for masses. Body weights and food and water consumption were measured daily, and at the end of the 2-week observation period a macroscopic examination was performed.

Clinical test parameters. Blood samples for hematology and clinical chemistry evaluation were obtained from the retroorbital plexus from animals under light anesthesia induced by CO_2 inhalation. EDTA was used as an anticoagulant for hematology samples, and lithium heparin was used as an anticoagulant for clinical chemistry samples. Blood samples were collected from animals before treatment and at the end of the study period (day 15). Food was withheld for approximately 18 h before blood collection, and samples were collected early in the working day to reduce biological variation; water was provided ad libitum. Clinical pathology parameters (hematological and clinical biochemical) were evaluated. Most hematology variables were evaluated with a Coul-

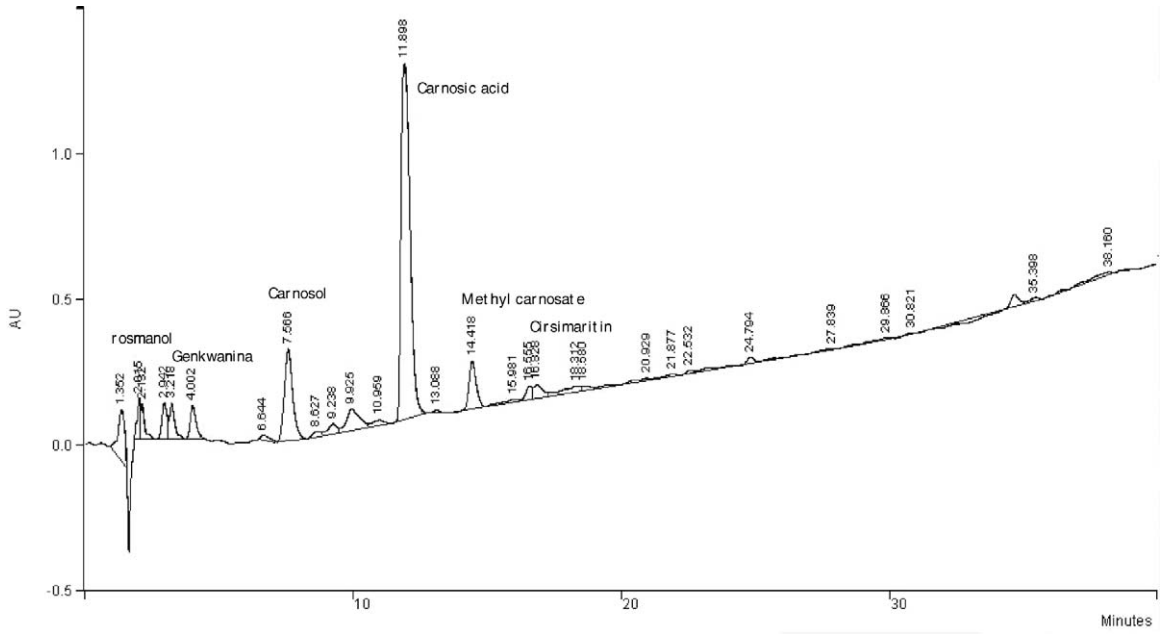

FIGURE 1. *High-performance liquid chromatogram of rosemary extract A.*

ter/CELL-DYN 3500 whole blood automated analyzer (Abbott, Chicago, Ill.). Differential leukocyte count and blood cell morphology slides were prepared with a Geometric Data Hemastainer (Thermofisher Scientific, Waltham, Mass.). Clinical chemistry parameters were evaluated with a spectrophotometer (Advia 1650 chemistry analyzer, Bayer, Leverkusen, Germany).

Anatomical pathology. At the end of the observation period (2 weeks), all animals were weighed, euthanized by CO_2 inhalation, exsanguinated, and necropsied. The necropsy included a macroscopic examination of the external surface of the body, all orifices, the cranial cavity; the brain and spinal cord, the nasal cavity and paranasal sinuses, and the thoracic, abdominal, and pelvic cavities and viscera. Descriptions of all macroscopic abnormalities were recorded. Samples of the following tissues and organs were collected from all animals at necropsy and fixed in neutral phosphate-buffered 4% formaldehyde solution: adrenal glands, brain, heart, ileum, jejunum, cecum, colon, duodenum, kidneys, liver, lungs, pancreas, spleen, stomach, testes, thymus, and thyroid and parathyroid glands. The organ:body weight ratios were calculated. All organ and tissue samples for histopathological examination were processed, embedded in paraffin, cut at an approximate thickness of 2 to 4 μm, and stained with hematoxylin and eosin. Slides of all organs and tissues listed above were collected on day 15 from the control and treated groups.

Statistical analyses. All data are expressed as means ± standard error of the mean (SEM) of 12 determinations (i.e., 12 samples each from six male and six female rats). Differences between control and treated groups were evaluated with a one-way analysis of variance followed by Dunnett's test *(8),* and differences were considered significant at $P < 0.05$.

TABLE 1. *Components of supercritical rosemary extracts analyzed by HPLC*

Compound	t_R (min)	% area	
		Extract A	Extract B
Rosmanol	2.942	2.7	3.9
Rosmanol isomer	3.218	2.7	0.6
Genkwanin	4.002	2.9	2.8
Carnosol	7.566	11.3	10.1
Carnosic acid	11.898	47.2	53.1
Methyl carnosate	14.418	5.2	6.3
Cirsimaritin	16.555	1.3	3.5
Not identified		26.7	19.7

RESULTS

Characterization and antioxidant potential of rosemary extracts. Two supercritical fluid extracts of rosemary, A and B, were used in the safety study. Both extracts were obtained with supercritical carbon dioxide and were characterized by HPLC to determine the concentration of phenolic diterpenes (see Fig. 1), and their antioxidant activity was evaluated with the DPPH method. The HPLC–diode array detection method was chosen based on previous work done in our laboratory *(23),* and compounds were characterized for their retention times (t_R) and UV spectra. Both extracts had similar relative amounts of phenolic components, as expressed in area percentage (Table 1), whereas the total concentration, expressed as micrograms per milligram of extract, for both the most active components (i.e., carnosol and carnosic acid) and the total phenolic diterpenes was around twice as high in extract A (Table 2).

TABLE 2. *Concentrations of main antioxidants in tested extracts and antioxidant activity (EC_{50})*

Compound	Extract A	Extract B
Carnosol (μg/mg)	112.7	45.9
Carnosic acid (μg/mg)	477.8	245.9
Total diterpenes (μg/mg)	700.1	343.1
EC_{50} (μg/ml)	6.32	15.82

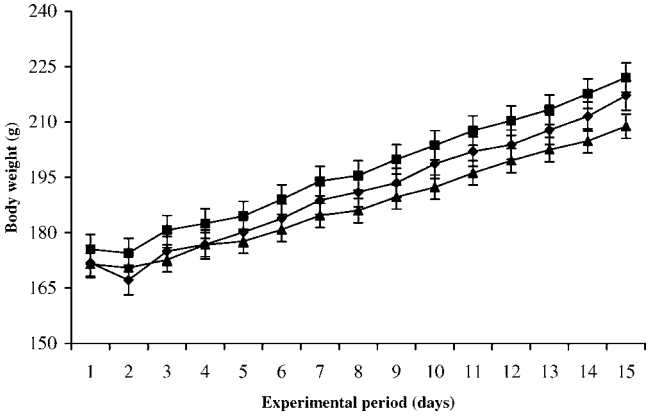

FIGURE 2. *Changes in body weight of rats during the 2-week observation period after treatment with rosemary extracts A and B. Group 1, control (♦); group 2, single oral dose of rosemary extract A at 2,000 mg/kg of body weight (■); group 3, single oral dose of rosemary extract B at 2,000 mg/kg of body weight (▲). Data represent the mean ± SEM for 12 rats.*

Antioxidant activity of the rosemary extracts was measured using the DPPH method. Both extracts had antioxidant activity, as demonstrated by the low EC_{50} values (less than 16 μg/ml), although the antioxidant activity found for extract A was much higher (EC_{50} = 6.32 μg/ml) because of the higher concentration of carnosic acid and total diterpenes than those in extract B (EC_{50} = 15.82 μg/ml). This result was as expected because an important relationship between the antioxidant activity of rosemary extracts and the concentration of such phenolic compounds was found in previous studies *(7).*

Acute safety study results. No abnormal signs, behavioral changes, body weight changes, macroscopic findings, or organ weight changes were observed. All animals survived the 2-week observation period. No body weight changes or anatomical abnormalities were noted at necropsy. Therefore, rosemary extracts A and B have a low risk of acute toxicity, and the oral lethal dosage (LD_{50}) for male and female rats is higher than 2,000 mg/kg of body weight.

Body weight and food consumption. Body weight data are depicted in Figure 2. There were no significant differences in body weights among groups. Similarly, no significant differences in food and water consumption were noted. Body weight and food and water consumption thus were unaffected ($P < 0.05$) by administration of rosemary extracts A and B as a single oral dose of 2,000 mg/kg of body weight.

Clinical pathology. No changes in hematological and clinical chemistry parameters of the treated rats were related to the corn oil treatment. The hematological and clinical chemistry parameters assessed 2 weeks after administration of A and B rosemary extracts as a single oral dose of 2,000 mg/kg of body weight were not significantly different compared with those of controls (Tables 3 and 4). No treatment-related changes were noted.

Anatomical pathology. Significant differences in organ weight or tissue:body weight ratios were related to the extracts. A and B rosemary extracts were not associated with any incidence of macroscopic and microscopic changes. No treatment-related histopathological changes were observed 2 weeks after adminstration of A and B rosemary extracts as a single oral dose of 2,000 mg/kg of body weight, and no histological correlates for the organ weight changes were found. Microscopically, minimal spaces in the periportal areas of the liver were observed occasionally

TABLE 3. *Hematological parameters in rats after the 2-week observation period following a single oral dose of rosemary extracts A and B at 2,000 mg/kg of body weight* [a]

Parameter[b]	Group 1 (control)	Group 2 (extract A)	Group 3 (extract B)
RBC ($\times 10^6/\mu l$)	7.86 ± 0.07	7.86 ± 0.07	8.06 ± 0.09
Hemoglobin (g/dl)	15.65 ± 0.13	15.45 ± 0.14	15.85 ± 0.12
Hematocrit (%)	47.35 ± 0.61	46.67 ± 0.30	47.89 ± 0.51
MCV (fl)	61.80 ± 0.55	60.60 ± 0.37	60.34 ± 0.37
MCH (pg)	19.95 ± 0.21	19.64 ± 0.18	20.02 ± 0.19
MCHC (g/dl)	33.73 ± 0.17	33.14 ± 0.28	33.46 ± 0.22
RDW (%)	18.96 ± 0.29	19.25 ± 0.16	18.59 ± 0.47
WBC ($\times 10^3/\mu l$)	8.67 ± 0.50	7.79 ± 0.53	8.27 ± 0.64
Banded neutrophils ($\times 10^3/\mu l$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Neutrophils ($\times 10^3/\mu l$)	1.05 ± 0.05	0.95 ± 0.05	1.20 ± 0.18
Eosinophils ($\times 10^3/\mu l$)	0.05 ± 0.01	0.04 ± 0.01	0.06 ± 0.01
Lymphocytes ($\times 10^3/\mu l$)	7.20 ± 0.46	6.54 ± 0.47	6.99 ± 0.50
Monocytes ($\times 10^3/\mu l$)	0.23 ± 0.03	0.19 ± 0.03	0.23 ± 0.05
Basophils ($\times 10^3/\mu l$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Platelets ($\times 10^3/\mu l$)	654.42 ± 13.81	652.50 ± 16.40	646.67 ± 29.52
MPV (fl)	6.30 ± 0.15	6.12 ± 0.12	6.17 ± 0.10
Platelets (%)	0.41 ± 0.02	0.41 ± 0.02	0.41 ± 0.02

[a] Data are expressed as mean ± SEM (n = 12; 6 male and 6 female rats) in each group. Differences between treatment groups and the control group were not significant.

[b] RBC, red blood cell count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, nucleated red blood cell count; WBC, white blood cell count; MPV, mean platelet volume.

TABLE 4. *Clinical chemistry parameters in rats after the 2-week observation period following a single oral dose of rosemary extracts A and B at 2,000 mg/kg of body weight*[a]

Parameter[b]	Group 1 (control)	Group 2 (extract A)	Group 3 (extract B)
Glucose (mg/dl)	77.17 ± 2.20	77.42 ± 2.81	78.00 ± 1.13
Urea nitrogen (mg/dl)	24.00 ± 1.42	25.75 ± 1.46	25.08 ± 1.00
Creatinine (mg/dl)	0.55 ± 0.01	0.55 ± 0.02	0.55 ± 0.03
Total protein (g/dl)	6.84 ± 0.13	6.91 ± 0.18	6.75 ± 0.12
Total bilirubin (mg/dl)	0.30 ± 0.03	0.34 ± 0.04	0.30 ± 0.02
Calcium (mg/dl)	11.12 ± 0.12	11.01 ± 0.12	10.87 ± 0.12
Sodium (mEq/liter)	150.75 ± 0.62	149.33 ± 0.79	149.50 ± 1.05
Potassium (mEq/liter)	6.37 ± 0.25	6.72 ± 0.20	6.89 ± 0.11
ASAT (U/liter)	97.00 ± 2.19	98.75 ± 1.34	98.75 ± 2.87
ALAT (U/liter)	49.33 ± 1.43	50.00 ± 2.26	50.58 ± 0.93
Alkaline phosphatase (U/liter)	100.25 ± 2.32	101.42 ± 2.73	100.42 ± 2.99

[a] Data are expressed as mean ± SEM ($n = 12$; 6 male and 6 female rats) in each group. Differences between treatment groups and the control group were not significant.
[b] ASAT, aspartate amino transferase; ALAT, alanine amino transferase.

in male rats from the treated and control groups. These spaces were considered incidental and not related to treatment.

DISCUSSION

There are no published conventional safety studies on rosemary extract despite widespread interest in its use because of its antioxidant activity. This study is the first published examination of the acute toxicity of rosemary leaf extracts. The objective was to evaluate the acute toxicity of two representative rosemary leaf extracts (A and B) and to provide information for further studies. The two extracts tested had different concentrations of phenolic diterpenes, representing medium and high values that can be found in commercial supercritical extracts. Extract A had higher antioxidant activity than did extract B. Antioxidant activity was closely related to the concentration of the phenolic constituents. Both extracts A and B had a low level of acute toxicity in Wistar rats. The oral lethal dose (LD_{50}) for male and female rats is greater than 2,000 mg/kg of body weight. Although rosemary extracts A and B did not produce acute toxicity, subchronic toxicity studies lasting 23 and 90 days are necessary to assess comprehensively the safety of these extracts.

Because the most significant human exposure to rosemary occurs through ingestion of this plant as a food additive, dosed feeding or the gavage route is the preferred route of exposure in rodent toxicity studies. However, the European Commission has approved the internal use of rosemary leaf for dyspeptic complaints and the external use as supportive therapy for rheumatic diseases and circulatory problems. Because of its anti-inflammatory and antioxidant properties, rosemary is used to treat localized injuries. Therefore, future studies on rodent chronic toxicity should be conducted using dermal exposure. This type of exposure is important because contact dermatitis through topical application of plants belonging to the Labiatae family has been described *(11, 19)*.

In the present study, rosemary extracts A and B were not associated with negative toxicological effects at a single dose of 2,000 mg/kg of body weight. No deaths or negative clinical signs were observed during the study. No significant differences in body weight gain, food and water consumption, organ weight, hematological parameters, clinical chemistry parameters, gross anatomy, or histological features were noted. These findings provide the basis for the selection of doses for use in long-term toxicity studies. These rosemary extracts were well tolerated by both male and female rats at an acute dose of 2,000 mg/kg of body weight.

ACKNOWLEDGMENTS

This work was supported by the Comunidad de Madrid and the Ministerio de Educación y Ciencia, projects S-0505/AGR/0153 and AGL2006-02031/ALI, and by Consolider Ingenio 2010 project CSD/2007/063.

REFERENCES

1. Aruoma, O. I., B. Halliwell, R. Aeschbach, and J. Loligers. 1992. Antioxidant and prooxidant properties of active rosemary constituents: carnosol and carnosic acid. *Xenobiotic* 22:257–268.
2. Barbut, S., D. B. Josphson, and A. J. Maurer. 1985. Antioxidant properties of rosemary oleoresin in turkey sausage. *J. Food Sci.* 50: 1356–1360.
3. Blumental, M., A. Goldberg, and J. Brinckmann (ed.). 2000. Herbal medicine. Expanded Commission E monographs. Herbs monographs, based on those created by a special expert committee of the German Federal Institute for Drugs and Medical Devices, 1st ed. American Botanical Council, Integrative Medicine Communications, Newton, Mass.
4. Brand-Williams, W., M. E. Cuvelier, and C. Berset. 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. Technol.* 28:25–30.
5. British Herbal Medicine Association. 1996. British herbal pharmacopeia, p. 162–163. British Herbal Medicine Association, Exeter, UK.
6. Bruneton, J. (ed.). 1995. Pharmacognosy, phytochemistry, medicinal plants, 2nd ed. Technique & documentation. Lavoisier, Paris.
7. Cavero, S., L. Jaime, P. J. Martín-Alvarez, F. J. Señoráns, G. Reglero, and E. Ibáñez. 2005. In vitro antioxidant analysis of SFE extracts from rosemary (*Rosmarinus officinalis* L.). *Eur. Food Res. Technol.* 221:478–486.
8. Dunnett, C. W. 1995. A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* 50:1096–1121.

9. European Scientific Cooperative on Phytotherapy. 1997. Monographs on the medicinal uses of plant drugs, vol. 3, p. 1–3. European Scientific Cooperative on Phytotherapy, Exeter, UK.
10. Frankel, E. N., S. W. Huang, R. Aeschbach, and E. Prior. 1996. Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinic acid, in bulk oil and oil-in-water emulsion. *J. Agric. Food Chem.* 44:131–135.
11. Gonzalez-Mahave, I., T. Lobesa, M. D. Del Pozo, A. Blasco, and M. Venturini. 2006. Rosemary contact dermatitis and cross-reactivity with other labiate plants. *Contact Dermatitis* 54:210–212.
12. Hoefler, C. F., J. Leurentin, F. Mortier, J. M. Pelt, and J. Guillemain. 1987. Comparative choleretic and hepatoprotective properties of young sprouts and total plant extract of *Rosmarinus officinalis* L. in rats. *J. Ethnopharmacol.* 19:133–143.
13. Huisman, M., H. L. Madsen, L. H. Skibsted, and G. Bertelsen. 1994. The combined effect of rosemary (*Rosmarinus officinalis* L.) and modified atmosphere packing as protection against warmed over flavoured in cooked minced pork meat. *Z. Lebensm.-Unters. -Forsch.* 198:57–61.
14. Ibáñez, E., A. Oca, G. de Murga, S. Lopez-Sebastian, J. Tabera, and G. Reglero. 1995. Supercritical fluid extraction and fractionation of different pre-processed rosemary plants. *J. Agric. Food Chem.* 47: 1400–1404.
15. Joyeux, M., A. Rolland, F. Mortier, and P. Dorfman. 1990. Terbutylhydroperoxide induced injury in isolated rat hepatocytes. A model for studying antihepatotoxic crude drugs. *Planta Med.* 56:171–174.
16. Kovar, K. A., B. Gropper, D. Freiss, and H. P. T. Ammon. 1987. Blood levels of 1,8 cineole and locomotor activity of mice after inhalation and oral administration of rosemary oil. *Planta Med.* 53: 315–318.
17. Lo, A. H., Y. C. Liang, S. Y. Lin-Shiau, C. T. Ho, and J. K. Lin. 2002. Carnosol, an antioxidant in rosemary, suppresses inducible nitric oxide synthase through down-regulating nuclear factor-κB in mouse macrophages. *Carcinogenesis* 23:983–991.
18. Mace, K., E. A. Offord, C. C. Harris, and A. M. Pfeifer. 1998. Development of in vitro models for cellular and molecular studies in toxicology and chemoprevention. *Arch. Toxicol. Suppl.* 20:227–236.
19. Martínez-González, M. C., J. J. Godoy Buján, W. Martínez Gómez, and E. Fonseca Capdevilla. 2007. Concomitant allergic contact dermatitis due to *Rosmarinus officinalis* (rosemary) and *Thymus vulgaris* (thyme). *Contact Dermatitis* 56:49–50.
20. Nakatani, N. 1992. Natural antioxidants from spices, p. 72–86. *In* M.-T. Huang, C.-T. Ho, and C. Y. Lee (ed.), Phenolic compounds in food and their effects on health. II. Antioxidants and cancer prevention. American Chemical Society, Washington, D.C.
21. Santoyo, S., S. Cavero, L. Jaime, E. Ibáñez, F. J. Señoráns, and G. Reglero. 2005. Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. essential oil obtained via supercritical fluid extraction. *J. Food Prot.* 68:790–795.
22. Schwarz, K., and W. Ternes. 1992. Antioxidative constituents of *Rosmarinus officinalis* and *Salvia officinalis*. *Z. Lebensm.-Unters. -Forsch.* 195:95–98.
23. Señorans, F. J., E. Ibáñez, S. Cavero, J. Tabera, and G. Reglero. 2000. Liquid chromatographic–mass spectrometric analysis of supercritical fluid extracts of rosemary plants. *J. Chromatogr. A* 870: 491–499.
24. Señorans, F. J., E. Ibáñez, and A. Cifuentes. 2003. New trends in food processing. *Crit. Rev. Food Sci. Nutr.* 43:507–526.
25. Starvic, B. 1994. Antimutagens and anticarcinogens in food. *Food Chem. Toxicol.* 32:79–90.
26. Taeto, F., and M. Fellin. 1988. *Rosmarinus officinalis* L. Extract production. Antioxidant and antimutagenic activity. *Perfum. Flavor.* 13: 48–54.
27. World Health Organization. 1987. Principles for the safety assessment of food additives and contaminants in food. Environmental Healthy Criterion 70. World Health Organization, Geneva.