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Effects of *Plantago ovata* Husk on Levodopa (with Carbidopa) Bioavailability in Rabbits with Autonomic Gastrointestinal Disorders

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ABSTRACT:

Gastrointestinal dysfunction is common in Parkinson's disease. Fiber therapy could be used to reduce the symptoms of gastrointestinal motility disorders. In a previous study, we showed that slowed gastrointestinal motility modified levodopa pharmacokinetics: area under the plasma concentration-time curve (AUC) and maximum plasma concentration (C_{max}) decreased and the elimination was delayed. In this study, we evaluated whether or not the hydrosoluble fiber *Plantago ovata* husk is useful in improving levodopa pharmacokinetics in rabbits with autonomic gastrointestinal disorders induced by the administration of the anticholinergic biperiden. Levodopa + carbidopa (20:5 mg/kg), biperiden (100 μg/kg), and *P. ovata* husk (at two different doses: 100 and 400 mg/kg) were administered orally to rabbits for two periods of time (7 or 14 days). In all groups of animals, the AUC values were approximately 50% higher on the final day of treatment than on day 1. C_{max} was also higher, with the greater increase at the 400 mg/kg dose of fiber, which resulted in a boost of approximately 35%. On day 1 of treatment and with both doses of fiber, AUC values were very similar to those obtained in previous work in rabbits with normal gastrointestinal motility, but the C_{max} was lower. However, after 7 or 14 days, the AUC values were higher, but C_{max} remained lower. The greatest differences were observed in plasma concentration before drug administration (C_{min}), for which the highest increase was obtained with the dose of 400 mg/kg fiber on day 14 of treatment (349.8%). *P. ovata* husk could be beneficial in patients with Parkinson's disease because it regulates stool transit in the intestine and because it improves levodopa pharmacokinetics when gastrointestinal peristalsis is slowed. These changes could lead to a possible delay in the onset of dyskinesias and to changes in prognosis.

There is growing recognition that one of the most frequent autonomic disorders in patients with Parkinson's disease is gastrointestinal dysfunction (Singer et al., 1992). In a detailed survey, Edwards et al. (1992) compared the frequency of gastrointestinal symptoms in patients with this disease and identified the most common symptoms as abnormal salivation, dysphagia, nausea, constipation, and defecatory dysfunction.

Constipation has been reported in more than 50% of patients with Parkinson's disease who attended a movement disorders clinic (Edwards et al., 1991). In elderly individuals, constipation may correlate with decreased physical activity, a low fluid intake, a diet poor in fiber, a sedentary life style, and the illness itself (Müller-Lissner et al., 2005).

Levodopa is the most effective antiparkinsonian drug available and is usually administered with an inhibitor of dopa decarboxylase such as carbidopa or benserazide. It is administered orally, and, therefore, its rate of absorption from the gastrointestinal tract has an important influence on its clinical effect. Stomach emptying rate and gastrointestinal motility are important factors affecting the rate and extent of drug absorption. Thus, a slow stomach emptying rate and slowed motility may delay levodopa absorption and reduce its clinical effectiveness. Levodopa may also delay gastric emptying, causing a vicious cycle that impairs compliance with therapy (Djaldetti et al., 1995; Koller and Rueda, 1998).

Fiber therapy could be used to reduce the symptoms of gastrointestinal motility disorders because fiber intake regulates stool transit in the small and large intestines. In patients with Parkinson's disease, daily fiber intake is frequently below the recommended daily allowance (Block et al., 1986). However, food intake decreases the absorption of levodopa and, therefore, its clinical effectiveness.

In a previous study (unpublished data), we showed that slowed intestinal motility caused by the administration of the anticholinergic biperiden diminished the amount of levodopa absorbed, with a decrease in the values of AUC and C_{max}. After several days of gastrointestinal motility reduction, AUC increased slightly to values near those obtained in normal rabbits, and C_{max} remained low.

As a continuation, in this study we evaluated the effects of the hydrosoluble fiber *Plantago ovata* husk on levodopa pharmacokinet-

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ABBREVIATIONS: AUC, area under the plasma concentration-time curve; C_{max}, maximum plasma concentration; C_{min}, plasma concentration before drug administration; t_{max}, time to reach maximum plasma concentration; F, fraction of dose absorbed; Cl, total body clearance; V_{ss}, volume of distribution in the steady state; AUMC, area under the first moment plasma concentration-time curve; MRT, mean residence time; λ, noncompartmental apparent first-order elimination rate constant; $t_{1/2\lambda}$, half-life associated with λ phase; V_a, apparent volume of distribution.

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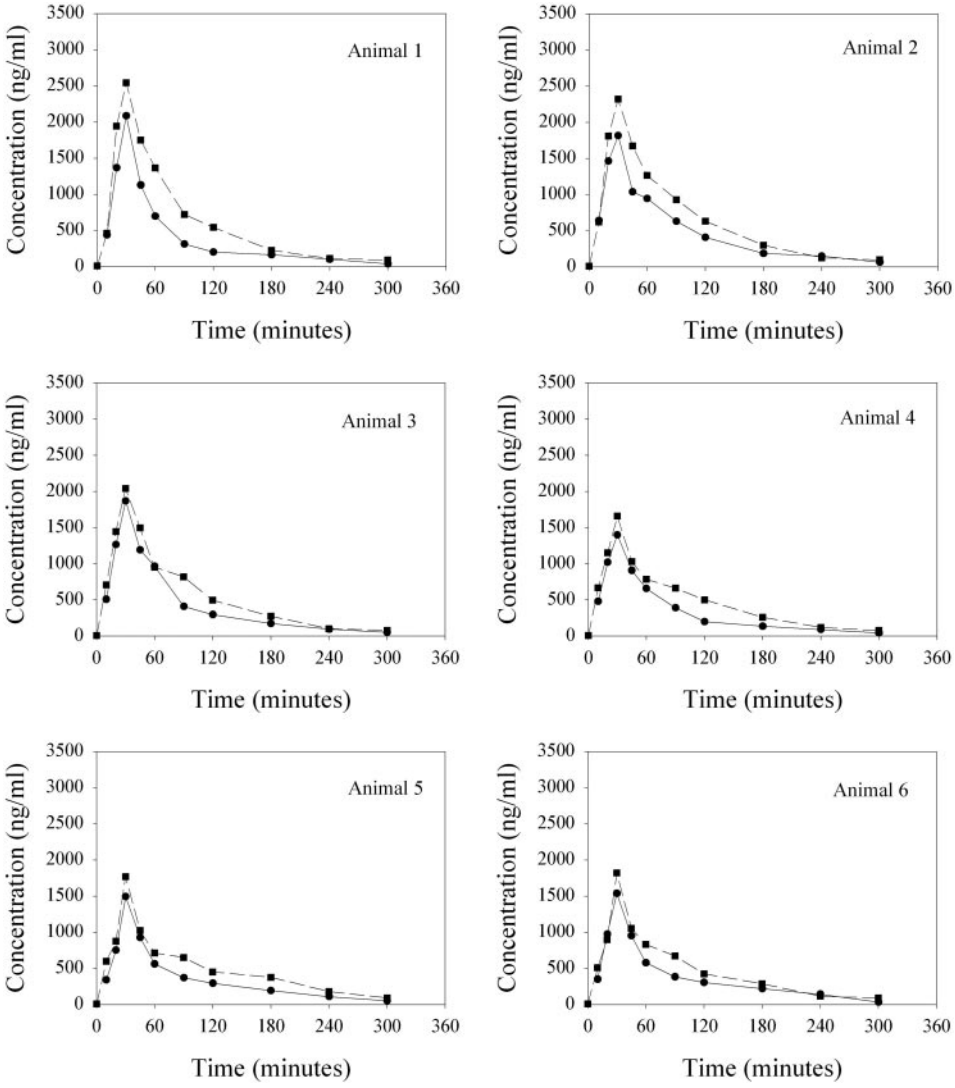

FIG. 1. Individual plasma concentrations of levodopa in rabbits after oral administration of 20:5 mg/kg levodopa + carbidopa with biperiden (100 μg/kg) and *P. ovata* husk (100 mg/kg) for 7 days (●——●, day 1; ■– –■, day 7).

ics (administered with carbidopa) in rabbits, again administering biperiden to slow gastrointestinal motility. The treatment lasted for two different periods of time (7 and 14 days) to verify the stabilization of levodopa concentrations and the gastrointestinal fiber effect. *P. ovata* husk was administered at two different doses of 100 and 400 mg/kg to allow evaluation of whether pharmacokinetic modifications are fiber dose-dependent.

Materials and Methods

We used 24 healthy, New Zealand White rabbits with a body weight range of 2.85 to 3.32 kg. The animals were housed in individual metal cages, which allowed the isolation of feces in a lower container and avoidance of coprophagia, under controlled conditions of temperature (19 ± 2°C), humidity (55 ± 10%), and light/dark cycle (12 h/12 h). The rabbits were maintained under these conditions for at least 1 week before the assay, with free access to water and standard laboratory chow.

Animals were randomly divided into different groups of six rabbits each. All animals received orally administered levodopa and carbidopa (Sinemet; Bristol-Myers Squibb Co., Madrid, Spain) and biperiden (Akineton; Abbott Laboratories, Madrid, Spain) dispersed in water and *P. ovata* husk (Plantaben; Madaus, Barcelona, Spain) immediately before drug administration. The different treatments were as follows: group 1, 20:5 mg/kg levodopa + carbidopa and biperiden (100 μg/kg) and *P. ovata* husk (100 mg/kg) for 7 days; group 2, 20:5 mg/kg levodopa + carbidopa and biperiden (100 μg/kg) and *P. ovata* husk (100 mg/kg) for 14 days; group 3, 20:5 mg/kg levodopa + carbidopa and biperiden (100 μg/kg) and *P. ovata* husk (400 mg/kg) for 7 days; and group 4, 20:5 mg/kg levodopa + carbidopa and biperiden (100 μg/kg) and *P. ovata* husk (400 mg/kg) for 14 days.

Drug administration was achieved via gastric intubation every morning at the same hour. A total of 50 ml of water was used for administration and cannula cleaning.

On the first day (day 1) and the last day (day 7 or 14, respectively) of treatment, levodopa concentrations were determined at different sampling times. Blood samples (3 ml) were collected through the cannula into heparinized containers, before and at 5, 10, 20, 30, 60, 90, 120, 180, 240, and 300 min after levodopa + carbidopa and biperiden and fiber oral administration. Two more blood samples were obtained from the marginal ear vein before (C_{min}) and at 20 min after drug administration (C_{max}) on days 3 and 5 when the study duration was 7 days and on days 3, 6, 9, and 11 when the duration was 14 days.

Blood samples were obtained from the left carotid artery previously cannulated with a silicone catheter (Silastic medical grade tubing, 1.02 mm inner diameter × 2.16 mm outer diameter). The catheters were placed with the animal under anesthesia with sodium pentobarbital (30 mg/kg i.v.). Drugs were administered after animals recovered totally from anesthesia.

Immediately after collection, plasma was separated by centrifugation and stored at −20°C until analysis. Levodopa extraction from plasma samples was carried out by using a catecholamine kit (Chromsystems Instruments & Chemicals GmbH, München, Germany) and was quantified by HPLC with electrochemical detection following the method described by Cummings et al. in 1990, slightly modified.

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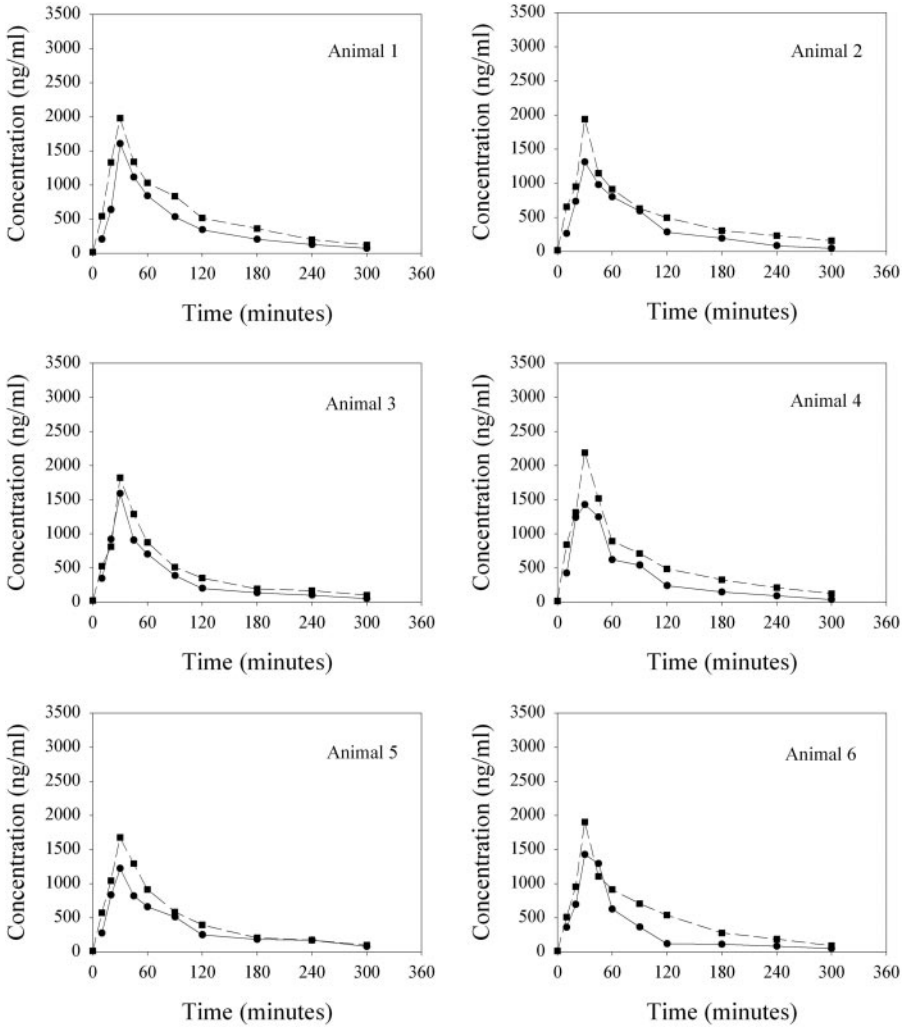

FIG. 2. Individual plasma concentrations of levodopa in rabbits after oral administration of 20:5 mg/kg levodopa + carbidopa with biperiden (100 μg/kg) and *P. ovata* husk (400 mg/kg) for 7 days (●——●, day 1; ■– –■, day 7).

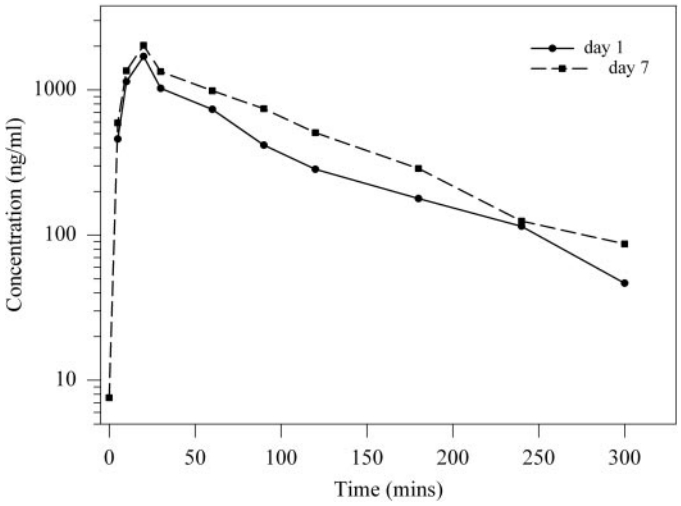

FIG. 3. Mean plasma concentrations of levodopa in rabbits after oral administration of 20:5 mg/kg levodopa + carbidopa with biperiden (100 μg/kg) and *P. ovata* husk (100 mg/kg) for 7 days.

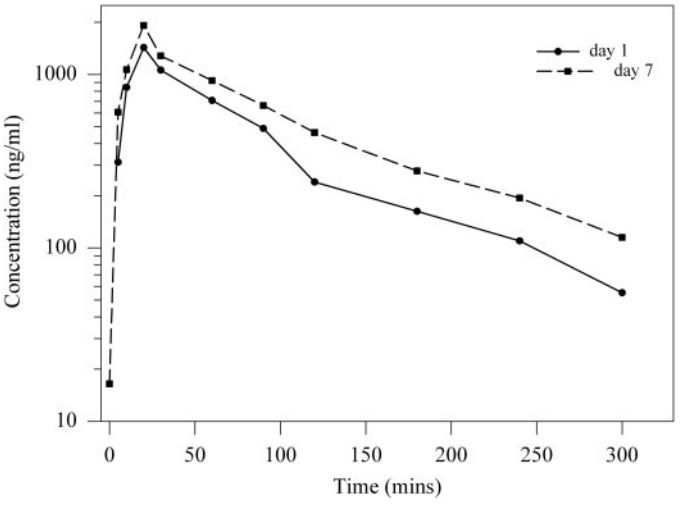

FIG. 4. Mean plasma concentrations of levodopa in rabbits after oral administration of 20:5 mg/kg levodopa + carbidopa with biperiden (100 μg/kg) and *P. ovata* husk (400 mg/kg) for 7 days.

The mobile phase consisted of 50 mM sodium dihydrogenphosphate buffer adjusted to pH 2.9 with 1 M orthophosphoric acid containing 250 mg/l heptanesulfonic acid and 80 mg/l EDTA and methanol (90:10, v/v). This mobile phase was pumped at a flow rate of 1 ml/min.

The analytical column was a 25 cm × 4.6 mm i.d. stainless steel column, packed with Spherisorb ODS-2 (5 pm particle size; Waters Chromatography SA, Madrid, Spain), and the potential applied was 500 mV. Interday and intraday accuracy and precision were within 10%, and neither heparin nor pentobarbital interfered with the assay.

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TABLE 1

Pharmacokinetic parameters obtained by noncompartmental analysis in rabbits after oral administration of 20:5 mg/kg levodopa + carbidopa with biperiden (100 μg/kg) and P. ovata husk (100 mg/kg) for 7 and 14 days

Parameters	Day 1 of 7		Day 7		Day 1 of 14		Day 14	
	Mean ± S.D.	CV	Mean ± S.D.	CV	Mean ± S.D.	CV	Mean ± S.D.	CV
		%		%		%		%
λ (min^{-1})	0.0118 ± 0.0019	16.17	0.0091 ± 0.0013	14.77	0.0099 ± 0.0019	19.05	0.0071 ± 0.0011	15.51
AUC ($\mu g \cdot min \cdot ml^{-1}$)[a,c]	114.58 ± 16.34	14.26	171.67 ± 25.91	15.09	119.14 ± 14.11	11.84	174.13 ± 9.71	5.57
C_{max} (μg/ml)[a,c]	1.70 ± 0.27	15.61	2.03 ± 0.35	17.06	1.54 ± 0.33	21.27	1.99 ± 0.12	6.26
t_{max} (min)	20.00		20.00		20.00		20.00	
Cl/*F* ($l \cdot kg^{-1} \cdot min^{-1}$)[a,d]	171.25 ± 22.94	13.39	118.65 ± 17.07	14.39	169.72 ± 18.81	11.09	115.16 ± 6.39	5.55
V_a/F (l/kg)	14.78 ± 2.82	19.12	13.08 ± 1.80	13.79	17.72 ± 4.26	24.04	16.61 ± 3.10	18.68
V_{ss}/F (l/kg)[a,c]	15.34 ± 2.50	16.31	12.27 ± 2.50	20.40	16.69 ± 3.41	20.44	13.77 ± 2.14	15.55
$t_{1/2\lambda}$ (min)	60.10 ± 9.23	15.37	77.05 ± 10.00	12.98	72.04 ± 14.04	19.49	99.33 ± 15.23	15.33
AUMC ($\mu g \cdot min^2 \cdot ml^{-1}$)[b]	10,639.5 ± 1882.7	17.70	17,498.1 ± 1781.3	10.18	11,674.8 ± 2122.2	18.18	20,659.1 ± 1664.5	8.06
MRT (min)[c]	89.58 ± 7.59	8.47	102.67 ± 7.33	7.14	98.15 ± 15.01	15.30	119.14 ± 13.38	11.23
MAT (min)	62.62		75.71		71.19		92.18	
F (%)	97.98		146.80		101.88		148.91	

CV, coefficient of variation; MAT, mean absorption time.
[a] Significant differences between day 1 and day 7 (*t* test, $P \leq 0.05$).
[b] Significant differences between day 1 and day 7 (Wilcoxon test, $P \leq 0.05$).
[c] Significant differences between day 1 and day 14 (*t* test, $P \leq 0.05$).
[d] Significant differences between day 1 and day 14 (Wilcoxon test, $P \leq 0.05$).

Pharmacokinetic Studies. Pharmacokinetic analysis was performed based on a noncompartmental description of the data observed. The WinNonlin computer program (Pharsight, Mountain View, CA) and formulae described by Gibaldi and Perrier (1982) were used to calculate the model-independent pharmacokinetic parameters. Maximum plasma levodopa concentration (C_{max}) and the time to reach maximum concentration (t_{max}) were read directly from the individual plasma concentration-time curves. The fraction of levodopa absorbed [*F* (percent)] was calculated by dividing the mean AUC by the value of the mean intravenous AUC obtained in a previous study (Diez et al., 2008) after levodopa administration alone.

Statistical Evaluation. All pharmacokinetic parameters were calculated for each animal, and the data are presented as the arithmetic mean ± S.D. Data were analyzed using the skewness test to determine the normality and the Cochran test to determine variance uniformity. When the data were normal and there was uniformity in the variance, *t* tests were used to evaluate differences between days 1 and 7 and between days 1 and 14. When the data were not normal or there was not uniformity in the variance, Wilcoxon's test was used. To evaluate differences in C_{min} and C_{max}, analysis of variance was performed, and Duncan's test was used to determine differences between data sets. When these data were not normally distributed or the variances were unequal, the Kruskal-Wallis test was used; when the results were significant, Wilcoxon's test was used to assess differences between data sets. A value of $P \leq 0.05$ was used as the level of significance for all analyses.

Results

7-Day Treatment. Levodopa individual concentrations as a function of time obtained on days 1 and 7 after oral administration of 20:5 mg/kg levodopa + carbidopa and biperiden (100 μg/kg) and *P. ovata* husk (100 or 400 mg/kg) are shown in Figs. 1 and 2, respectively. Figures 3 and 4 show the plots of mean plasma levodopa concentrations obtained for the two groups.

The noncompartmental pharmacokinetic parameters obtained for levodopa are summarized in Tables 1 (100 mg/kg *P. ovata* husk) and 2 (400 mg/kg *P. ovata* husk). Table 3 gives the C_{min} and C_{max} values obtained for the different groups.

When levodopa + carbidopa with biperiden was administered with 100 mg/kg *P. ovata* husk for 7 days (group 1), the AUC value obtained for levodopa increased 49.8% from day 1 (171.67 $\mu g \cdot min \cdot ml^{-1}$) to day 7 (114.58 $\mu g \cdot min \cdot ml^{-1}$) (significant differences), and C_{max} increased 19.4% (1.70 versus 2.03 μg/ml) (significant differences). The values obtained for C_{min} increased progressively (30.5% from day 3 to day 7) with the duration of the treatment (significant differences were found between days 3 and 5 and in a comparison of days 3 and 5 with day 7, respectively). Significant differences were also found between day 1 and day 7 for Cl/*F*, V_{ss}/F, AUMC, and MRT. The increase in MRT and decrease in Cl/*F* indicated that levodopa remained in the body longer.

When levodopa + carbidopa with biperiden was administered with 400 mg/kg *P. ovata* husk for 7 days (group 3), the increase in AUC was 49.9% after 7 days of treatment (114.31 $\mu g \cdot min \cdot ml^{-1}$ on day 1 and 171.33 $\mu g \cdot min \cdot ml^{-1}$ on day 7) (significant differences). This increment was almost the same as that obtained with the lower dose of fiber. The increase observed in C_{max} (1.43 μg/ml on day 1 and 1.92 μg/ml on day 7; significant differences) was 34.3%. This value was similar to that obtained after administration of 100 mg/kg for 14 days. The increase in C_{min} (66.9% from day 3 to day 7, significant differences between day 7 and days 3 and 5, respectively) was 2-fold that observed with the low dose of fiber. As was found with the previous two groups, significant differences were identified for Cl/*F* and V_{ss}/F, and levodopa elimination was also slower as treatment progressed, as can be deduced from the reduction in Cl/*F* and the increase in MRT.

14-Day Treatment. Levodopa individual concentrations as a function of time obtained on days 1 and 14 after oral administration of 20:5 mg/kg levodopa + carbidopa with biperiden (100 μg/kg) and 100 or 400 mg/kg *P. ovata* husk are shown in Figs. 5 and 6, respectively. Figures 7 and 8 show the plots of mean plasma levodopa concentrations obtained for the two groups.

As in the previous groups, the noncompartmental pharmacokinetic parameters obtained for levodopa are summarized in Tables 1 (100 mg/kg *P. ovata* husk) and 2 (400 mg/kg *P. ovata* husk). Table 3 gives the C_{min} and C_{max} values.

When the 100 mg/kg dose of *P. ovata* husk was used for a 14-day treatment (group 2), the AUC increase (119.14 $\mu g \cdot min \cdot ml^{-1}$ on day 1 and 174.13 $\mu g \cdot min \cdot ml^{-1}$ on day 14) was 46.2% (significant differences), and, as observed with group 1 above, the C_{max} increase (1.54 μg/ml on day 1 and 1.99 μg/ml on day 14) was slightly lower (29.2%, significant differences). C_{min} also increased with treatment duration to be 109.6% higher on day 14 compared with day 3; significant differences were found between all days except between day 11 and day 14. Significant differences were also found between

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TABLE 2

Pharmacokinetic parameters obtained by noncompartmental analysis in rabbits after oral administration of 20:5 mg/kg levodopa + carbidopa with biperiden (100 μg/kg) and P. ovata husk (400 mg/kg) for 7 and 14 days

Parameters	Day 1 of 7		Day 7		Day 1 of 14		Day 14	
	Mean ± S.D.	CV	Mean ± S.D.	CV	Mean ± S.D.	CV	Mean ± S.D.	CV
		%		%		%		%
λ (min^{-1})	0.0094 ± 0.0017	17.89	0.0079 ± 0.0014	18.24	0.0083 ± 0.0024	28.57	0.0084 ± 0.0013	15.24
AUC ($\mu g \cdot min \cdot ml^{-1}$)[a,b]	114.31 ± 10.02	8.77	171.33 ± 17.81	10.40	114.28 ± 20.38	17.84	182.44 ± 28.60	15.68
C_{max} ($\mu g/ml$)[a,b]	1.43 ± 0.15	10.58	1.92 ± 0.17	8.93	1.50 ± 0.25	16.61	1.99 ± 0.17	8.42
t_{max} (min)	20.00		20.00		20.00		20.00	
Cl/F ($l \cdot kg^{-1} \cdot min^{-1}$)[a,b]	176.08 ± 15.27	8.67	117.81 ± 12.44	10.56	180.07 ± 34.33	19.06	111.91 ± 17.52	15.65
V_a/F (l/kg)[b]	19.41 ± 4.30	22.16	15.42 ± 3.42	22.18	22.78 ± 5.25	23.03	13.61 ± 3.05	22.42
V_{ss}/F (l/kg)[a,b]	17.26 ± 2.32	13.44	14.15 ± 1.83	12.93	22.73 ± 5.18	22.81	13.61 ± 3.07	22.53
$t_{1/2\lambda}$ (min)	76.07 ± 12.90	16.96	90.53 ± 16.33	18.04	90.52 ± 29.00	32.03	84.03 ± 13.60	16.19
AUMC ($\mu g \cdot min^2 \cdot ml^{-1}$)[a,b]	8717.6 ± 1416.2	16.25	20,707.8 ± 3775.1	18.23	12,094.3 ± 3755.4	31.05	21,574.6 ± 3890.5	18.03
MRT (min)	98.38 ± 13.64	13.86	120.51 ± 14.26	11.83	79.02 ± 4.99	6.32	118.69 ± 14.15	11.92
MAT (min)	71.42		93.55		52.06		91.73	
F (%)	97.75		146.51		97.73		156.01	

CV, coefficient of variation; MAT, mean absorption time.
[a] Significant differences between day 1 and day 7 (t test, $P \leq 0.05$).
[b] Significant differences between day 1 and day 14 (t test, $P \leq 0.05$).

TABLE 3

Values of C_{min} and C_{max} obtained after oral administration of 20:5 mg/kg levodopa + carbidopa with biperiden (100 μg/kg) and P. ovata husk (100 or 400 mg/kg) for 7 and 14 days to rabbits

	100 mg/kg		400 mg/kg	
	Mean ± S.D.	CV	Mean ± S.D.	CV
	ng/ml	%	*ng/ml*	%
C_{min} (0 min)				
Day 3	5.80 ± 1.57	27.08	9.86 ± 2.48	25.14
Day 5	6.67 ± 1.57[b]	23.48	12.53 ± 2.38	19.01
Day 7	7.57 ± 1.48[b,c]	19.56	16.46 ± 3.27[b,c]	19.84
C_{max} (20 min)				
Day 1	1666.56 ± 318.10	19.09	1430.57 ± 151.38	10.58
Day 3	1840.99 ± 410.78	22.31	1419.92 ± 282.63	19.90
Day 5	1756.83 ± 297.89	16.96	1627.03 ± 207.52	12.75
Day 7	2059.45 ± 409.31	19.87	1918.12 ± 171.29[a,b,c]	8.93
C_{min} (0 min)				
Day 3	6.75 ± 1.59	23.59	13.58 ± 4.04	29.77
Day 6	8.17 ± 3.19[e]	39.05	18.82 ± 3.88[e]	20.61
Day 9	9.73 ± 3.08[e,f]	31.66	26.98 ± 6.92[e,f]	25.64
Day 11	12.43 ± 3.57[e,f,g]	28.70	32.98 ± 6.55[e,f]	19.86
Day 14	14.15 ± 3.79[e,f,g]	26.79	39.45 ± 9.18[e,f,g]	23.28
C_{max} (20 min)				
Day 1	1541.89 ± 328.00	21.27	1498.16 ± 248.79	16.61
Day 3	1557.41 ± 286.64	18.40	1481.59 ± 379.52	25.62
Day 6	1661.81 ± 246.60	14.84	1619.95 ± 475.50	29.35
Day 9	1696.65 ± 145.87	8.60	1704.70 ± 498.08	29.22
Day 11	1895.37 ± 231.61[d,e,f]	12.22	1806.43 ± 448.93	24.85
Day 14	1986.52 ± 124.45[d,e]	6.26	1988.99 ± 167.40[d,e]	8.42

CV, coefficient of variation.
Significant differences: [a] with day 1; [b] with day 3; [c] with day 5 (Duncan's test, $P \leq 0.05$).
Significant differences: [d] with day 1; [e] with day 3; [f] with day 6; [g] with day 9 (Duncan's test, $P \leq 0.05$).

day 1 and day 14 for the remainder of the pharmacokinetic parameters, except for λ, $t_{1/2\lambda}$, and V_a/F. Changes in Cl/F and MRT were similar to those observed after 7 days of treatment and indicated that the time that levodopa remained in the body increased slightly with the duration of treatment.

After 14 days of treatment and with the highest dose of *P. ovata* husk (group 4), the increase in AUC (114.28 $\mu g \cdot min \cdot ml^{-1}$ on day 1 and 182.44 $\mu g \cdot min \cdot ml^{-1}$ on day 14) was 59.6% (significant differences). For C_{max} (1.50 $\mu g/ml$ on day 1 and 1.99 $\mu g/ml$ on day 14), the increase (32.7%; significant differences) was similar to that obtained after 7 days of treatment. There was a much higher increase in the value of C_{min}, which increased 190.5% from day 3 to day 14 after the administration of 400 mg/kg *P. ovata* husk, with significant differences between all days except between the values of days 9 and 11 and days 11 and 14. If we compare the values obtained for this parameter after 7 and 14 days of treatment, respectively, we can see that the value determined on day 14 was approximately 2.4-fold higher than that obtained on day 7 (Table 3).

For the remaining pharmacokinetic parameters, we found significant differences between day 1 and day 14 for AUMC, MRT, V_{ss}/F, V_a/F, and Cl/F. The elimination of levodopa was also slower on day 14, with a higher value of MRT and a lower value for Cl/F.

Comparison of Day 7 and Day 14. Comparing of values obtained on day 7 and day 14, we found no significant differences for any pharmacokinetic parameters. After the administration of 100 mg/kg fiber, the plasma concentrations of levodopa at the different sampling

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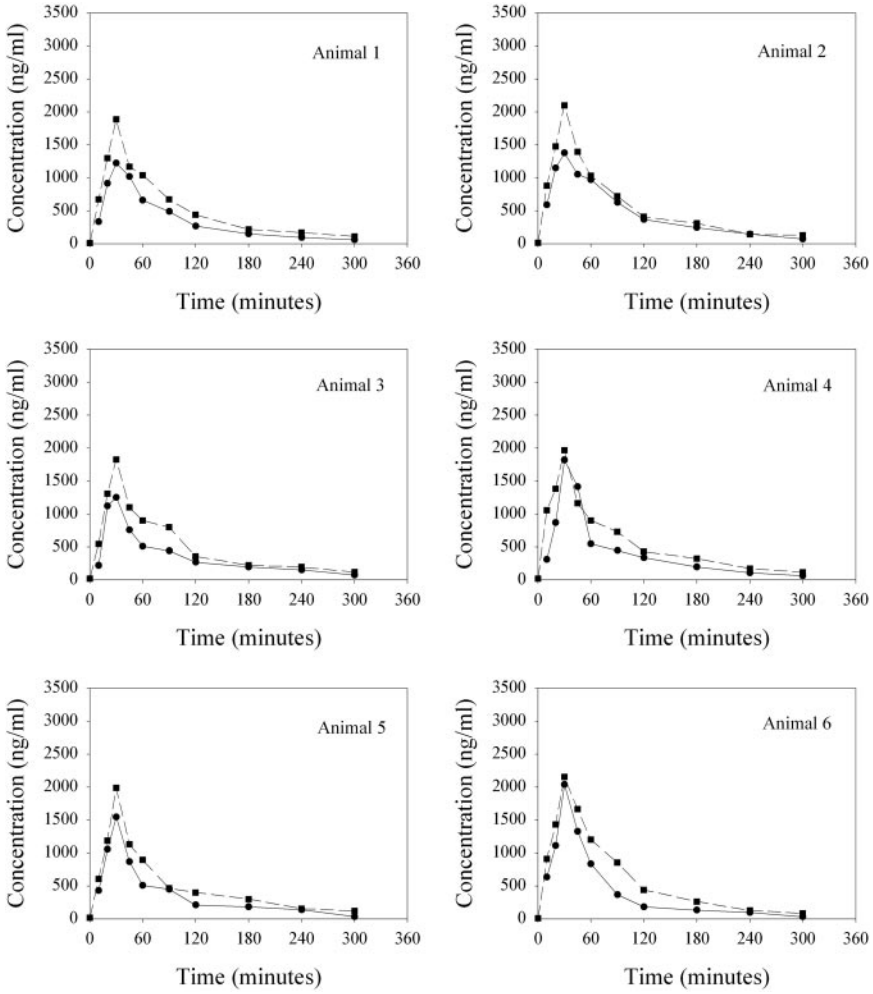

FIG. 5. Individual plasma concentrations of levodopa in rabbits after oral administration of 20:5 mg/kg levodopa + carbidopa with biperiden (100 μg/kg) and *P. ovata* husk (100 mg/kg) for 14 days (●——●, day 1; ■– –■, day 14).

times were similar after 14 days of treatment to those after 7 days, except at the final sampling time of 300 min, when levels were higher on day 14.

If the dose of fiber administered was 400 mg/kg, the concentrations of levodopa at the different sampling times were also higher on day 14 of treatment than on day 7 except for a single sampling time at which they were very similar: 240 min (188 versus 184 ng/ml).

Discussion

In this study, we evaluated how *P. ovata* husk, administered at two different doses of 100 or 400 mg/kg, modifies the bioavailability and other pharmacokinetic parameters of levodopa. We administered the levodopa with carbidopa to rabbits in which we induced impaired gastrointestinal motility via oral biperiden administration for different time periods (7 and 14 days).

At the lower dose of *P. ovata* husk, the time needed for AUC and C_{max} stabilization was 7 days, with similar mean values on days 7 and 14 of treatment. However, C_{min} stabilization did not occur until days 11 to 14.

In a previous study, we evaluated the pharmacokinetics of levodopa (administered with carbidopa) in rabbits with gastrointestinal motility slowed by biperiden administration (unpublished data) (see Table 4 for key pharmacokinetic parameters). Comparing these results with the obtained in this study, we can see that the administration of fiber to rabbits with slowed gastrointestinal peristalsis initially (day 1 of treatment) did not modify the AUC value. However, there was an increase in this value after 7 (18.8% with both doses of fiber) and 14 days (15.4% with 100 mg/kg fiber and 23.8% with 400 mg/kg fiber) of treatment. If we compare the value of C_{max}, we can see that it was slightly lower on day 1 with the administration of fiber in all groups than without the administration of *P. ovata*, although at the end of the treatments, the values obtained were very similar. The greatest differences were observed in C_{min}, which was also higher in the presence of fiber. The highest increase for this parameter was found with the dose of 400 mg/kg fiber on day 14 of treatment (8.77 and 39.45 ng/ml with and without fiber: 349.8% higher with fiber).

If we compare the results of the present work with those obtained in another previous study (Diez et al., 2008) performed with administration of *P. ovata* husk (100 and 400 mg/kg) to rabbits with normal gastrointestinal peristalsis, we can see that on day 1 and after the administration of the low dose of fiber (100 mg/kg), AUC values were very similar (114.58 versus 112.65 $\mu g \cdot min \cdot ml^{-1}$ on day 1 of 7 and 119.14 versus 108.37 $\mu g \cdot min \cdot ml^{-1}$ on day 1 of 14) and C_{max} values were lower (1.70 versus 2.02 μg/ml on day 1 of 7 and 1.54 versus 2.03 μg/ml on day 1 of 14). After 7 days of treatment, AUC (171.67 $\mu g \cdot min \cdot ml^{-1}$) was higher than that obtained in rabbits with normal gastrointestinal motility (141.30 $\mu g \cdot min \cdot ml^{-1}$), and C_{max} was lower (2.03 versus 2.21 μg/ml). On day 14, the results were similar: C_{max} was also lower (1.99 versus 2.30 μg/ml) and AUC was higher (174.13 versus 148.79 $\mu g \cdot min \cdot ml^{-1}$) in the presence of biperiden and fiber.

Regarding the use of 400 mg/kg *P. ovata* husk, the administration of the drugs to normal rabbits (Diez et al., 2008) yielded higher values

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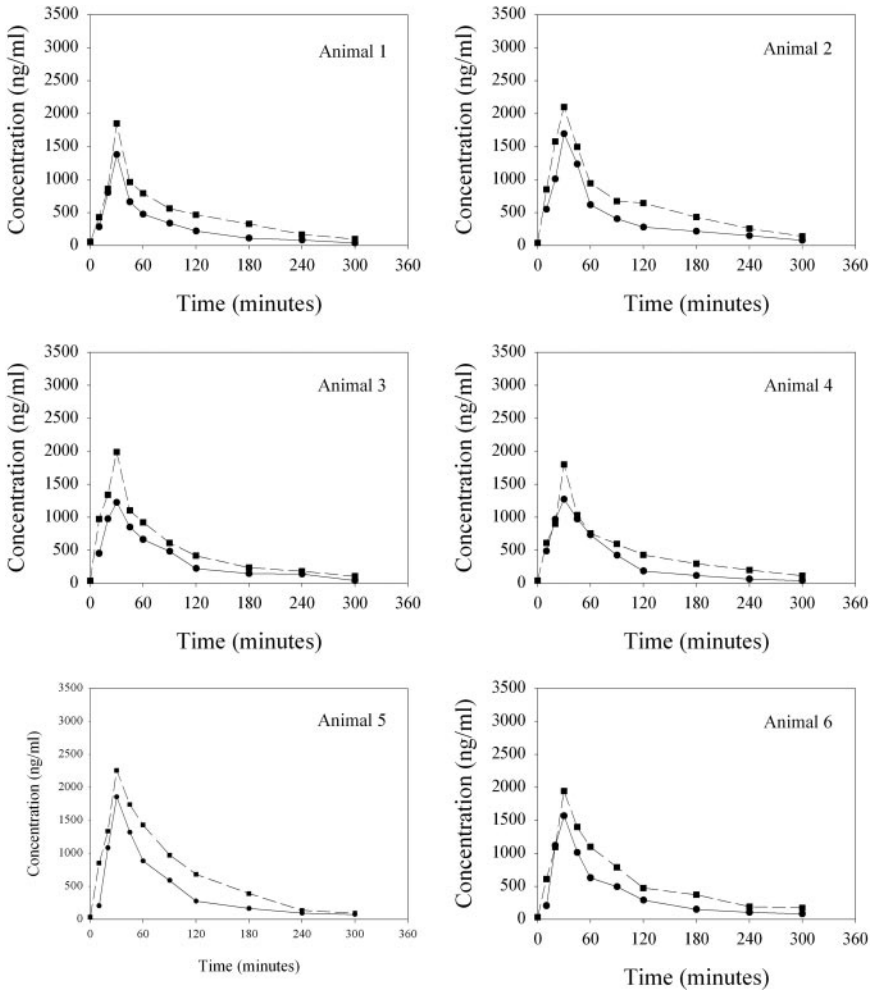

FIG. 6. Individual plasma concentrations of levodopa in rabbits after oral administration of 20:5 mg/kg levodopa + carbidopa with biperiden (100 μg/kg) and *P. ovata* husk (400 mg/kg) for 14 days (●——●, day 1; ■– –■, day 14).

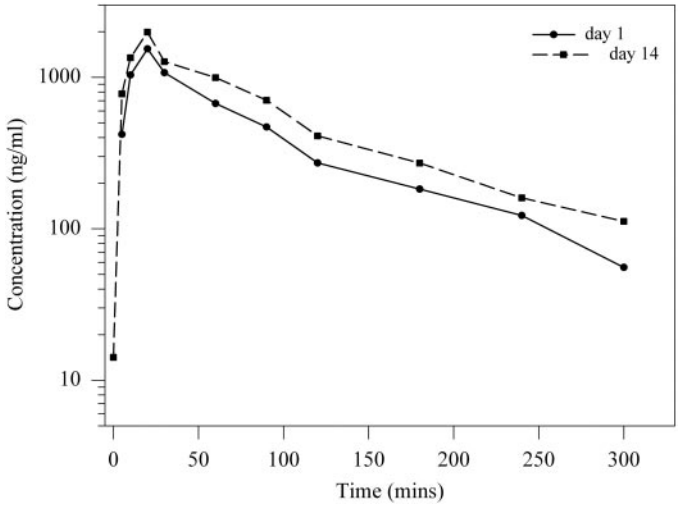

FIG. 7. Mean plasma concentrations of levodopa in rabbits after oral administration of 20:5 mg/kg levodopa + carbidopa with biperiden (100 μg/kg) and *P. ovata* husk (100 mg/kg) for 14 days.

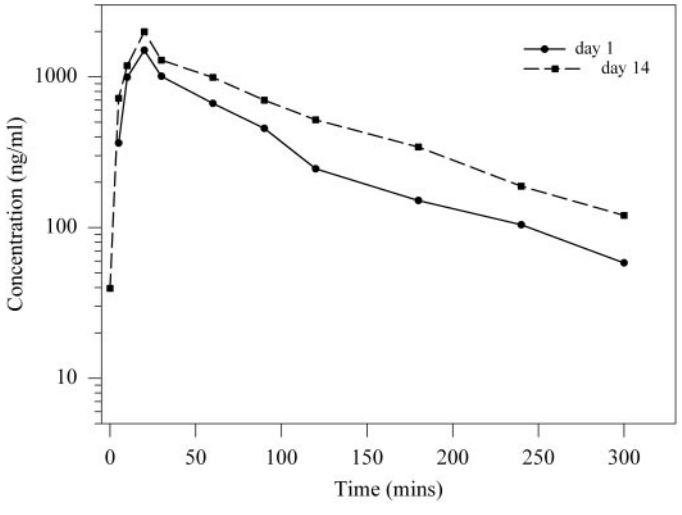

FIG. 8. Mean plasma concentrations of levodopa in rabbits after oral administration of 20:5 mg/kg levodopa + carbidopa with biperiden (100 μg/kg) and *P. ovata* husk (400 mg/kg) for 14 days.

for AUC and C_{max} on day 1 of treatment, compared with those in the present work (12.9 and 28.5%, respectively). However, after 7 days, C_{max} was higher (14.7%), but AUC was lower (7.1%).

When this high dose of fiber was administered for the 14-day period, the mean values obtained for AUC when biperiden was not administered (Diez et al., 2008) were slightly higher on day 1 (125.50 versus 114.28 $\mu g \cdot min \cdot ml^{-1}$) and lower on day 14 (167.19 versus 182.44 $\mu g \cdot min \cdot ml^{-1}$). On the other hand, C_{max} was lower in the presence of biperiden on days 1 (2.04 versus 1.50 μg/ml) and 14 (2.26 versus 1.99 μg/ml). Mean values obtained for AUC (171.33 and

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TABLE 4

Pharmacokinetic parameters obtained by noncompartmental analysis in rabbits after oral administration of 20:5 mg/kg levodopa + carbidopa and biperiden (100 μg/kg) for 7 days and 14 days

Data are arithmetic means.

Parameters	Day 1 of 7	Day 7	Day 1 of 14	Day 14
AUC ($\mu g \cdot min \cdot ml^{-1}$)	115.6	144.5	110.5	147.3
C_{max} ($\mu g/ml$)	1.73	1.99	1.72	1.94
C_{min} (ng/ml)		6.51		8.77

182.44 $\mu g \cdot min \cdot ml^{-1}$) and for C_{max} (1.92 and 1.99 $\mu g/ml$) with 400 mg/kg *P. ovata* husk were similar on days 7 and 14 after administration, as was the case with 100 mg/kg.

If we evaluate the influence of the fiber dose used and the treatment duration, we can see that AUC after 7 days was almost the same with 400 or 100 mg/kg *P. ovata* husk and that the values of C_{max} were 12.9% higher when the lowest dose of fiber was administered. After 14 days of treatment, AUC was 4.8% higher with 400 mg/kg, and C_{max} was the same. The results showed significant differences in almost all pharmacokinetic parameters between day 1 and day 7 or day 14 of treatment. However, there were no significant differences between day 7 and day 14 of treatment.

Slow gastric emptying (gastroparesis) and constipation are frequent in patients with Parkinson's disease (Edwards et al., 1991, 1992; Byrne et al., 1994; Quigley, 1996; Hardoff et al., 2001; Müller-Lissner et al., 2005). Several approaches to its treatment have been investigated. Drugs that block dopamine receptors accelerate gastric emptying, presumably via an effect on gastric dopamine receptors (McCallum, 1985) and may improve levodopa absorption. Metoclopramide hydrochloride speeds up gastric emptying but is contraindicated in Parkinson's disease because it readily gains access to the central nervous system and blocks striatal dopamine receptors, thus exacerbating parkinsonism. Results with domperidone are contradictory. Domperidone blocks dopamine receptors but does not cross the blood-brain barrier, and it has been reported that it can be used safely in Parkinson's disease and improves both gastric emptying, measured objectively, and symptoms of gastroparesis in patients with Parkinson's disease (Soykan et al., 1997). However, other authors (Lesser and Bateman, 1985) have indicated that it can exacerbate parkinsonism.

Cisapride acts as a prokinetic agent by stimulating acetylcholine release from myenteric cholinergic neurons (Wiseman and Faulds, 1994). This drug can also increase plasma concentrations of levodopa (Neira et al., 1995) and reduce levodopa dose failures (Djaldetti et al., 1995) in patients with Parkinson's disease, but concerns about potential cardiotoxicity have led to a ban on or severe restriction of the use of cisapride in many countries.

Another approach is fiber therapy, which could be used to reduce the symptoms of gastrointestinal motility disorders because it regulates stool transit in the small and large intestines. Ashraf et al. (1997) indicated that among patients with Parkinson's disease who had confirmed constipation, *P. ovata* husk increased stool frequency and weight but did not alter colonic transit or anorectal function. They concluded that *P. ovata* husk produces both subjective and objective improvements in constipation related to Parkinson's disease.

A possible problem is that *P. ovata* husk administration could delay gastric emptying and, consequently, delay levodopa absorption from the gastrointestinal tract and increase its presystemic elimination. The effect of fiber on gastric emptying is controversial. Benini et al. (1995) stated that fiber naturally present in food delays gastric emptying of a solid meal. With guar gum, another hydrosoluble fiber, gastric emptying (Harju et al., 1984) and intestinal transit were delayed (Schönfeld et al., 1997). According to Bergmann et al. (1992), the intake of 10.8 g of psyllium significantly delayed gastric emptying from the 3rd hour after a meal.

Conversely, Bianchi and Capurso (2002) indicated that inclusion of a single 5-g dose of dietary fiber (guar gum and ispaghula husk) with a solid standard meal did not influence gastric emptying and orocecal transit time. McIntyre et al. (1997) found that 7 g of ispaghula husk had no significant effect on gastric emptying of a rice pudding test meal. Russell and Bass (1984) indicated that meals with 3% psyllium and 1.5% guar gum did not modify the gastric emptying of solids, and Rigaud et al. (1998) observed no delay in the gastric emptying of the solid and liquid phases of a meal with psyllium (7.4 g).

The results obtained in our study may indicate that *P. ovata* husk does not modify gastric emptying, at least under our experimental conditions; taking into account the fact that the value of t_{max} was the same whether levodopa was administered alone or with the dietary fiber.

P. ovata husk can also retain part of the dose of levodopa administered. This would explain the fact that on the first day of treatment we obtained lower initial concentrations of levodopa when fiber was administered. Fiber can also retain part of the dose of carbidopa administered, diminishing its effectiveness to inhibit the enzyme aromatic amino acid decarboxylase initially. After several days of treatment, we obtained higher levels of levodopa, so we think that *P. ovata* husk, or any product of its partial hydrolysis, could inhibit the enzyme aromatic amino acid decarboxylase, as has been demonstrated for other enzymes (Isaksson et al., 1982; Leng-Peschlow, 1991), an effect that is more significant with the higher dose of fiber.

Astarloa et al. (1992), after the administration of an insoluble fiber, demonstrated a useful effect of this fiber on plasma levodopa concentration and motor function in patients with Parkinson's disease who have marked constipation. The greatest elevation of levodopa levels was found early (at 30 and 60 min) after oral administration. In the current study, with *P. ovata* husk the maximum levodopa plasma concentrations did not increase, but the final concentrations of the drug were higher and the decrease in concentration was thus less steep. This more modest decrease is very important to patients with Parkinson's disease because it could result in longer therapeutic effects of the antiparkinsonian drug. In addition, it results in less variation in plasma levels with a consequent decrease in motor fluctuations that are caused by high levodopa concentrations or by sharp decreases in these concentrations.

In conclusion, *P. ovata* husk could be beneficial in patients with Parkinson's disease because it regulates stool transit in the intestine and also because of the improvement that results in levodopa pharmacokinetics when gastrointestinal peristalsis is slowed. These changes would lead to a possible delay in the onset of dyskinesias and in a reduction of their effects.

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