Journal of the Neurological Sciences 365 (2016) 175–180

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Mucuna pruriens for Parkinson's disease: Low-cost preparation method, laboratory measures and pharmacokinetics profile

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ARTICLE INFO

Article history:
Received 28 January 2016
Received in revised form 17 March 2016
Accepted 3 April 2016
Available online 16 April 2016

Keywords:
Parkinson's disease
Mucuna pruriens
Levodopa

ABSTRACT

Background: Parkinson's disease (PD) is a progressive neurological condition. Levodopa (LD) is the gold standard therapy for PD patients. Most PD patients in low-income areas cannot afford long-term daily Levodopa therapy. The aim of our study was to investigate if *Mucuna pruriens* (MP), a legume with high LD content that grows in tropical regions worldwide, might be potential alternative for poor PD patients.
Methods: We analyzed 25 samples of MP from Africa, Latin America and Asia. We measured the content in LD in various MP preparations (dried, roasted, boiled). LD pharmacokinetics and motor response were recorded in four PD patients, comparing MP vs. LD + Dopa-Decarboxylase Inhibitor (DDCI) formulations.
Results: Median LD concentration in dried MP seeds was 5.29%; similar results were obtained in roasted powder samples (5.3%), while boiling reduced LD content up to 70%. Compared to LD + DDCI, MP extract at similar LD dose provided less clinical benefit, with a 3.5-fold lower median AUC.
Conclusion: Considering the lack of a DDCI, MP therapy may provide clinical benefit only when content of LD is at least 3.5-fold the standard LD + DDCI. If long-term MP proves to be safe and effective in controlled clinical trials, it may be a sustainable alternative therapy for PD in low-income countries.

1. Introduction

Mucuna pruriens variant *utilis* (MP) is a leguminous plant belonging to the Fabaceae family, which grows spontaneously in the tropical and subtropical areas of the world (Fig. 1). It is considered an invasive plant, as it grows rapidly without any particular measure needed to ensure its growth. Although MP has a high nutritional value due to its content of nutrients, antinutrients and biologically active compounds [1], it is not commonly used as food, but rather as a soil fertilizer [2]. The analysis of MP antinutrients revealed the presence of phytates, tannins, saponins, and compounds with potentially toxic properties, such as alkaloids [1,3].

Levodopa is the gold standard in the treatment of Parkinson's disease (PD). Levodopa was isolated for the first time from the legume *Vicia faba* in 1910, and its chemical structure was established definitively in 1913 [4]. However, MP had been used since ancient times in Ayurvedic medicine to treat symptoms of corresponding to PD [5] due to the high Levodopa concentration in its seeds. Levodopa was isolated from MP seeds for the first time in 1937 [6] and its concentration therein was estimated to be approximately 4–6% [7]. Preliminary studies carried out in parkinsonian rats [8], primates [9] and humans [10,11] suggest that extracts of MP may be used to improve PD motor symptoms, with a favorable pharmacokinetic profile that may account for a lower risk and severity of dyskinesias [8,9,11].

Despite being the cheapest treatment for PD, Levodopa therapy is not currently sustainable for thousands of PD patients with a low-income, because of limited availability and affordability. [12] National

Abbreviations: PD, Parkinson's disease; LD, Levodopa; MP, *Mucuna pruriens*; DDCI, dopa-decarboxylase inhibitor.

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http://dx.doi.org/10.1016/j.jns.2016.04.001

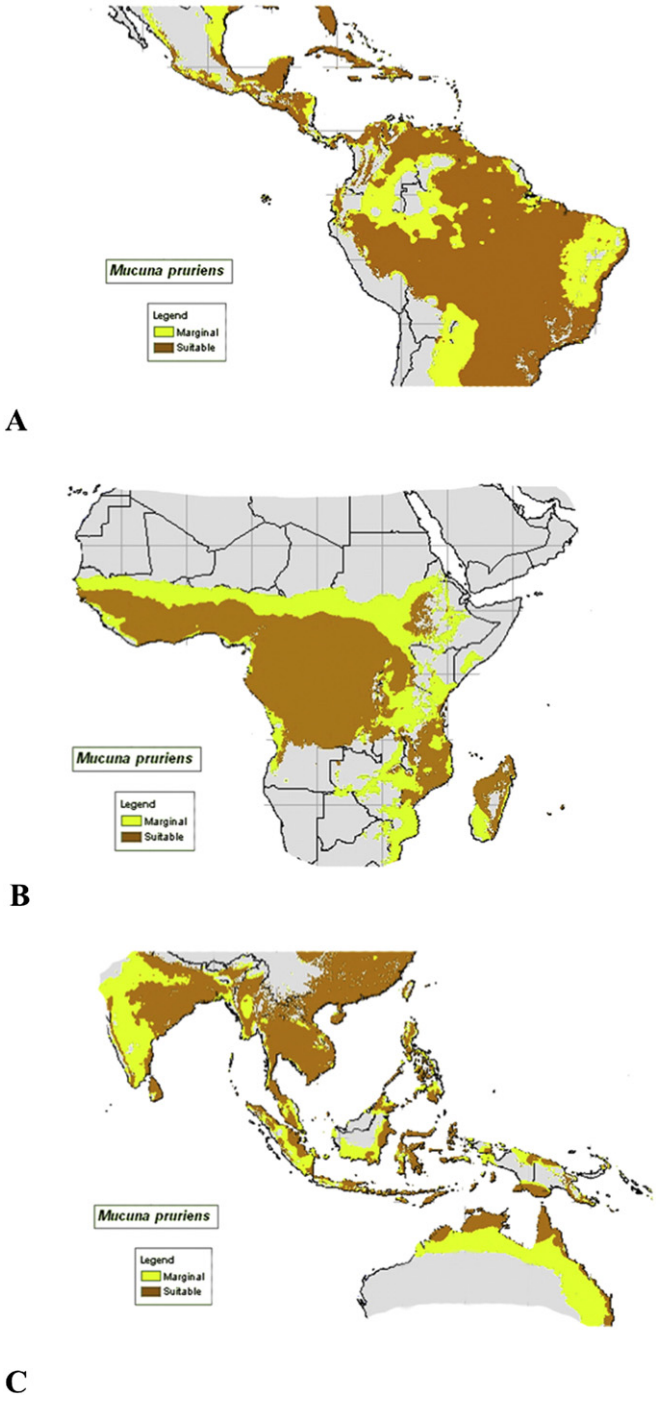

Fig. 1. Distribution of *Mucuna* growth worldwide (modified from http://www.discoverlife.org/mp/20q?search=Mucuna+pruriens&flags=glean:&mobile=close).

Health Insurance systems of most low-income countries do not cover any antiparkinsonian medication. In Sub-Saharan Africa, approximately 60% of the population lives with less than US$ 2/day and the daily cost of Levodopa treatment is about 1 US$/day i.e. approx. 50% of a patient's income [12]. On the contrary, the mean cost of 1 kg of MP seeds is about 1 US$/kg both in Ghana and Bolivia [Akpalu A., Laguna J. pers. comm.]. One kilogram of MP seeds should be enough for approximately 50 days of treatment, using an average daily dose. Nevertheless, it is too early for widespread therapeutic use of MP in patients with PD for a number of reasons. First, little information is available on the exact content in Levodopa and alkaloids of the various types of MP seeds in different regions of the world. To date, there are no practical indications on how many MP seeds are to be used to obtain an established amount of LD (e.g. number of MP dried seeds needed to obtain clinical response similar to 100 mg of Levodopa-based medications). In second instance, differences in cooking methods affect the content of LD in MP [13]. However, no indications are available on the safest cooking method and its impact on the concentration of LD and antinutrients. Indeed, MP may be toxic when large unrestricted quantities are ingested due to LD and, potentially, alkaloids [7,14]. Ghanaian literature suggests that fresh or dried MP seeds may be used in humans adopting a number of safety measures, including: eating no >10–15 seeds at a time; removing teguments; boiling the seeds for at least 40 min and throwing away the cooking water [15]. Obviously, more scientific measures are needed to assess the safety and efficacy of MP as potential long-term therapy for PD. Therefore, it is of utmost importance to provide reliable estimates of the content of LD and antinutrients in MP, because PD patients may self-medicate inappropriately if they misinterpret the information about MP that is currently available. Finally, previous animal and clinical studies comparing MP with standard LD formulations [8,11] suggested intrinsic dopa-decarboxylase inhibitor (DDCI)-like activity, but the substances responsible for this phenomenon have not been identified. Natural compounds with intrinsic DDCI activity have been described, one of which (named genistein) has been found in MP seeds [16]. However, the finding of genistein in MP seeds has never been replicated so far.

In the present study, we collected various ecotypes of MP collected in various areas of the world to measure their content of Levodopa, potentially toxic anti-nutrients (i.e. alkaloids) and the DDCI genistein. In addition, we investigated Levodopa plasma concentration and clinical response in four PD patients who self-medicated using MP.

2. Materials and methods

During the 2012–2014 period, we collected 25 samples of *M. pruriens* (Table 1). All the samples were examined and identified as MP seeds by expert botanists. In MP samples, the concentrations of Levodopa, alkaloids, a compound with intrinsic DDCI activity (named genistein) and its precursor (i.e. genistin) were determined using high performance liquid chromatography together with mass spectrometry (HPLC-MS) according to standard methods (available upon request).

2.1. Measurement of Levodopa concentration

2.1.1. Dried seeds

The samples of raw seeds were prepared by grinding about 1 g of seeds finely in a mortar. The resulting powder was suspended in 50 mL of acid water (containing hydrochloric acid, 0.01 N) and extracted with the help of ultrasound for 24 h. At the end of the extraction procedure, the aqueous extract was filtered and 1 mL aliquot was collected and analyzed by HPLC-MS. Besides Levodopa, this technique detected and quantified the following compounds: *dopamine*, *adrenaline*, *noradrenaline*, *serotonin*, *retrorsine*, *crotaline and cytisine*.

2.1.2. Cooked seeds (boiling vs. roasting)

Further measurements of Levodopa concentrations were performed after standardized cooking methods in selected samples of MP seeds. We focused our analysis on the seeds purchased from authorized merchants in Togo and Bolivia, because these varieties can be easily bought by patients (compared to the samples provided by the University of Accra or the USDA; Table 1).

a) *BOILING.* Standardized boiling (soaked for 15 h + boiled for 90 min) with different seed weight/soaking water ratios. The water used for cooking and soaking was also analyzed.
b) *ROASTING.* Roasting in a stove at 150° for 15 min, with subsequent decortication. Whole seeds, decorticated seeds, and teguments were analyzed separately. The samples of roasted seeds were prepared by weighing about 1 g of the sample and grinding it finely in

Table 1
Quantification of Levodopa in *Mucuna pruriens* samples according to preparation (results in mass%).

N°	Code	Details[a]	Country–continent		Source[b]	Preparation[c]	Levodopa content (%)
1	Local ecotype	White	Togo	Africa	Market	Dried seed	5.69
						Boiled (3 volumes)	1.69
						Boiled (4 volumes)	1.86
						Boiled (5 volumes)	0.81
2	Local ecotype	Black	Togo	Africa	Market	Dried seed	6.41
						Boiled (3 volumes)	2.35
						Boiled (4 volumes)	1.52
						Boiled (5 volumes)	2.55
3	Local ecotype	Black	Kumasi, Ghana	Africa	Market	Dried seed	6.47
4	GH1739	White	Accra, Ghana	Africa	University	Dried seed	1.29
5	GH1740	White	Accra, Ghana	Africa	University	Dried seed	3.27
6	GH1741	White	Accra, Ghana	Africa	University	Dried seed	5.79
7	GH1742	White	Accra, Ghana	Africa	University	Dried seed	4.47
8	GH1743	White	Accra, Ghana	Africa	University	Dried seed	6.09
9	GH1744	Mottled	Accra, Ghana	Africa	University	Dried seed	4.04
10	GH1745	White	Accra, Ghana	Africa	University	Dried seed	3.76
11	GH1746	Mottled	Accra, Ghana	Africa	University	Dried seed	8.34
12	GH1747	White	Accra, Ghana	Africa	University	Dried seed	5.29
13	GH3957	Mottled	Accra, Ghana	Africa	University	Dried seed	4.75
14	GH3972	White	Accra, Ghana	Africa	University	Dried seed	1.21
15	GH3973	Black	Accra, Ghana	Africa	University	Dried seed	6.26
16	PI227479	Mottled	Costa Rica	Latin America	USDA	Dried seed	5.84
17	PI337098	Local name Velvet bean	Brazil	Latin America	USDA	Dried seed	4.56
18	PI364362	Local name Velvet bean	Mozambique	Africa	USDA	Dried seed	4.89
19	PI365411	Local name Velvet bean	Mozambique	Africa	USDA	Dried seed	4.46
20	PI365415	Local name Velvet bean	Mozambique	Africa	USDA	Dried seed	4.53
21	PI365414	Local name Velvet bean	Mozambique	Africa	USDA	Dried seed	3.52
22	PI494877	Color ash. Local name Coffee.	Zambia	Africa	USDA	Dried seed	7.49
23	Local ecotype	Black	Santa Cruz, Bolivia	Latin America	Market	Dried seed	6.57
						Roasted powder	5.7
24	Local ecotype	White	Santa Cruz, Bolivia	Latin America	Market	Dried seed	9.49
						Roasted powder	5.04
25	Local ecotype		India	Asia	Drug store in Germany	Roasted powder	5.3

Abbreviations: USDA, ARS. Plant Genetic Resources Conservation Unit. Griffin, Georgia, USA.
[a] Include color and/or USDA Plantid code details.
[b] The Germplasm from the USDA is being distributed by the U.S. National Plant Germoplasm System (NPGS) for education, agricultural research or breeding purposes.
[c] Inhibition was carried out using three volumes of deionized water for each weight unit of the dried seeds. Several volumes of deionized water were added to the imbibed seeds vs. the initial dried seed weight for cooking.

a mortar. The resulting powder was processed and analyzed using the same method as described above.

2.2. Measurement of alkaloids and genistein concentration

2.2.1. Alkaloids

The analyses were carried out by gas chromatography-mass spectrometry (GC-MS) and by gas chromatography-Thermal Energy Analysis (GC-TEA), a selective detector of molecules containing nitrogen atoms. The following alkaloids were investigated: 7-cyano-1,2,3,4-tetrahydroisoquinoline, N-methylcytisine, sparteine and lupanine. The identification and quantification of these analytes were carried out by using standard methods available on the market and by constructing calibration curves according to the method of the external reference standard. The analyses carried out by GC-TEA in search of nitrogenous compounds were carried out on the same extracts in dichloromethane analyzed by GC-MS. The compounds that generated a detectable signal were quantified as lupanine, constructing a calibration curve. The analyses performed by GC-MS and GC-TEA were performed using equipment settings that enabled a direct comparison between retention times in order to achieve correspondence between the signals recorded by GC-MS and peaks recorded by GC-TEA. Specifically, capillary columns having the same type of stationary phase were used and the same thermal ramp was set up.

Genistein and its precursor genistin were measured in dried seeds and roasted powder of Bolivian MP samples, roasted powder from Indian samples (samples 23 and 25 in Table 1), and marketed MP-derived extracts (Table 2). The samples of roasted seeds were prepared according to the procedure described above and analyzed by HPLC-MS.

2.3. Levodopa plasma concentrations and pharmacodynamics

During 2013–2014, four patients with idiopathic PD were examined at our Institute, who reported to self-medicate with MP, either alone ($n = 2$) or together with standard anti-PD therapy ($n = 2$). Three male patients were regularly purchasing and taking MP extract in capsules three-to-four times daily (each capsule contained 300 mg of MP-derived powder) manufactured in Italian licensed drugstores from raw material obtained in India. A fourth female patient with motor fluctuations and dyskinesias was taking MP powder from seeds she had obtained by herself in Bolivia (Table 2). We measured plasma Levodopa concentrations at fixed time-points over a 3-h period after intake of Levodopa + DDCI (either carbidopa or benserazide) or MP powder in the morning on two consecutive days, after overnight withdrawal of usual anti-PD therapy (at least 12 h). Acute Levodopa challenge dose was calculated according to the patient's usual fasting first morning dose. Measured pharmacokinetic variables included time to peak (t_{max}), peak plasma Levodopa concentration (C_{max}) and the area under the plasma concentration–time curve (AUC) [17]. The Unified Parkinson's Disease Rating scale motor score (UPDRS-III) and the Abnormal Involuntary Movements Scale (AIMS) were used to assess motor response and dyskinesias, respectively. The study protocol was carried out in compliance with the Declaration of Helsinki and its further amendments. Written informed consent was obtained from all patients and all subjects included in video.

Table 2
Levodopa plasma pharmacokinetic measures and pharmacodynamic response.

Features		Patient 1	Patient 2	Patient 3	Patient 4[a]
Gender		M	M	M	F
Age (years)		85	69	62	52
Disease duration (years)		8	6	4	10
Body weight		48.5	73	72	68
UPDRS part III – Off		29	22	10	28
Hoehn and Yahr stage – Off		2	2	1	3
Levodopa daily dose (mg/day)		300	400	300	475
Concomitant DA agonists (type, mg/day)		–	ROP, 6 mg	–	ROP, 16 mg
Duration of chronic MP therapy (years)		2	2	2	0.5
Levodopa + DDCI challenge					
Levodopa dose (mg)		150	150	150	100
Weight adjusted Levodopa dose (mg/kg)		3.2	2.1	2.1	1.5
Motor response	UPDRS III at 90 min	21	12	8	11
	Change vs. OFF (%)	27.6	45.4	20.0	60.7
	UPDRS III at 180 min[b]	–	–	–	22
	AIMS at 90 min	0	0	0	10
	C_{max} (μg/mL)	2.43	3.5	5.7	0.92
	t_{max} (min)	45	30	15	45
	AUC (μg/mL) × min	212.0	241.1	239.6	55.3
Mucuna pruriens challenge					
Levodopa content in MP (%)[c]		15	15	15	5.7
Levodopa content in MP (mg)		135	180	180	400
Weight-adjusted Levodopa content (mg/kg)		2.8	2.5	2.5	5.9
Levodopa ratio MP:LD + DDCI		0.9: 1	1.2: 1	1.2: 1	4: 1
Motor response	UPDRS III at 90 min	24	18	8	10
	Change vs. OFF (%)	17	18	20	63.6
	Change vs. LD + DDCI at 90 min[d]	−10%	−27%	0%	+3%
	UPDRS III at 180 min[b]	–	–	–	11
	AIMS at 90 min	0	0	0	5
LD pharmacokinetics	C_{max} (μg/mL)	1.82	1.2	2.05	1.53
	t_{max} (min)	45	30	15	30
	AUC (μg/mL) × min	98.4	65.2	86.8	95.5
	AUC ratio LD + DDCI:MP[e]	1.96	4.64	3.47	0.58

Abbreviations: AUC, Area under the plasma concentration-time curve; C_{max}, peak plasma levodopa concentration; DDCI, Dopa decarboxylase inhibitor (i.e. benserazide or carbidopa); LD, Levodopa; t_{max}, time to peak plasma levodopa concentration; UPDRS III, Unified Parkinson's Disease Rating Scale, motor examination.

[a] Considering the median ratio between the AUC on LD + DDCI vs. MP (namely LD without DDCI) was 3.5, the MP dose in this patient was calculated using a 1:4 ratio.

[b] UPDRS III was performed at 180 min only in patient#4.

[c] MP powder daily amount patients used to take at home before the baseline visit. Levodopa content in each sample was calculated. Patients#1 to #3 were assuming MP extract manufactured in Italian licensed drugstores from raw material obtained in India. Patient#4 was assuming MP powder obtained from roasted seeds (Table 1, sample 23).

[d] Calculated as [(UPDRS III at 90 min on MP – UPDRS III at 90 min on LD + DDCI) / UPDRS III in OFF] * 100.

[e] Adjusted for Levodopa dose 150 mg.

3. Results

3.1. Measurement of Levodopa concentration

All results of laboratory analyses are summarized in Table 1.

3.1.1. Dried seeds

HPLC-MS analyses detected only Levodopa. The other compounds sought by this technique were not found in any of the samples analyzed (<10 mg/kg in seed samples and <2 mg/L in water samples). Median [25-75IQR] concentration of Levodopa in the powder of roasted seeds was 5.29% [4.5–6.3]. Levodopa concentration was verified also correcting for protein content.

3.1.2. Boiled seeds

Results of imbibition tests are reported in Table 1. No Levodopa was found in cooking or imbibition water. Median [25-75IQR] Levodopa concentration after boiling was reduced by 70.3% [68.6–70.4] compared to dried seeds.

3.1.3. Roasted seeds

Median [25-75IQR] concentration of Levodopa in the powder of roasted seeds was 5.3% [5.2–5.5], similar to raw dried seeds. We herewith describe the preparation method followed by a Bolivian neurologist (J.L.) with a long-standing clinical experience in the use of MP seeds in the treatment of PD: (a) roasting in a pan for 15 min; (b) waiting for all the teguments to burst (the noise resembles popcorn bursting); (c) removing from the fire and hulling; (d) roasted seeds may be crunched or ground in a little coffee grinder, passed through a sieve and added to soup or water as a powder (Supplementary video).

3.1.4. Marketed extract of MP

The concentration of Levodopa in the MP-derived extract that patients purchase and take home as capsules was 15% (corresponding to 45 mg of Levodopa for each 300 mg capsule).

3.2. Measurement of alkaloids, genistein and genistin concentrations

3.2.1. Alkaloids

The search was carried out on all the seeds available (raw and dried), as well as on the boiled seeds, cooking water, imbibition water and roasted seeds. No alkaloids were found either by GC-MS or by HPLC-MS. GC-MS detected a series of peaks that could have been generated by compounds with isoquinoline, pyrrolidine and steroid nuclei. These peaks were semi-quantified using a lupanine external standard. GC-TEA analyses generated chromatograms that showed peaks due to the presence of nitrogenous compounds that could not be identified, but that are reasonably generated by alkaloids. However, in view of the high complexity of the matrixes analyzed and of the multiplicity of nitrogenous compounds that they could contain, it was not possible to establish with certainty whether a substance detected actually was an alkaloid or not in the absence of the specific reference standard.

3.2.2. Genistein/genistin

We did not find either genistein or genistin in all the MP samples tested, including marketed samples.

3.3. Levodopa plasma pharmacokinetic and pharmacodynamic measures

In the three PD patients assuming marketed extracts of MP, the median [25-75IQR] ratio between the AUC obtained from 150 mg Levodopa + Benserazide and the AUC obtained with the MP extract was 3.47[2.7–4.0] (Table 2). Motor response at 90 min and 180 min from intake of MP extract was variable, ranging from 27% worsening to approx. the same extent of improvement in UPDRS-III scores versus an equal amount of dispersible Levodopa + Benserazide (Table 2). No dyskinesias were recorded in these patients (they have never described any dyskinesias so far).

A fourth female patient with advanced PD and a history of motor fluctuations and dyskinesias reported that she was taking MP powder (from seeds she had obtained in Bolivia) on top of her standard anti-PD therapy. Considering the median 3.5-fold lower AUC measure obtained comparing marketed MP powder with Levodopa + Benserazide, we administered MP powder calculating a 4-fold Levodopa content. In this case, the explorative 4:1 ratio also considered the approximate 5-fold lower bioavailability of Levodopa in the central nervous system without a DDCI [11,18]. Compared to Levodopa + DDCI, MP induced a similar reduction in UPDRS-III scores at 90 min, but considerably better motor performance at 180 min, due to a 30-min longer duration of ON condition (180 min vs. 210 min, respectively) with reduced dyskinesias (Table 2).

4. Discussion

All the types of MP seeds (*utilis* variant) we analyzed from different countries worldwide contain Levodopa, with a median percentage of 5.3%. We did not identify a more promising ecotype, hence the most easily available ecotype in the various geographical areas may be used. Nonetheless, the wide range of concentrations (from 1.2% to 9.5%) does not allow an a priori estimate of Levodopa content in individual MP samples. These findings highlight an important take-home message of the present study: the therapeutic dose required to treat PD patients must be based on an a priori measurement of Levodopa concentration in the specific MP ecotype to avoid unpleasant side effects.

Overall, no known alkaloids were detected in all the MP samples tested. This finding supports the safety of long-term consumption of MP, when daily dose is controlled [7–9,19]. However, it is certainly advisable to eat MP after cooking to reduce the ingestion of the antinutrients that usually occur in raw legumes (phytic acid, tannins, saponins, etc.) and after removing its teguments, to avoid side effects due to fermentation.

Another issue addressed in the present study was the potential difference in Levodopa concentration between dried MP seeds and different cooking methods. Compared to dried seeds, boiling greatly reduced the concentration of Levodopa in the seeds, while roasting seem to play a minor effect on the concentration of Levodopa. On the other hand, roasting seems to be a better cooking method than boiling for a number of other reasons: (a) it is easier to standardize, as it avoids the need to measure the amount of cooking/soaking water, the measurement of temperature and cooking time; (b) it facilitates tegument removal (teguments contain antinutrients); (c) it just requires a pan and a little grinder (that can be replaced by a mortar); (d) roasted powder is safe from a hygienic point of view; (e) it is easy to store even for a long time.

Levodopa is unaffordable in most low-income regions of the world [12], it is therefore mandatory to identify an interventional strategy designed to ease the economic burden of pharmacological management of PD in developing countries. Levodopa-based strategy using MP seeds may well fit this objective. However, this means investigating the safety and efficacy of dosage regimens in order to minimize side effects and tailor them to the needs of the patient. In tropical areas, the cost of home preparation of MP roasted powder is negligible and it is easy to store for a long time. Levodopa-based therapy using MP may be considered a potential alternative therapy for PD in developing countries, because it is very easy to find at local markets and easy to grow in all tropical areas, with a low-cost preparation method. On the other hand, marketed capsules of MP extract in many developed countries and/or on the web do not meet these requirements, because they have elevated production costs and overall low mean LD content.

A final issue concerning MP relates to clinical effects in patients with PD. Preclinical and clinical studies suggest substantial effects of MP in the improvement of parkinsonian features with reduced dyskinesias compared to standard Levodopa + DDCI formulations [8,9,11,20,21]. These effects were thought to be associated with a more favorable pharmacokinetic profile and with potential intrinsic DDCI-like activity [8,9]. In the final part of this study, we investigated Levodopa plasma concentrations and motor response in a small number of patients (who were already taking MP at home to overcome obvious ethical issues) in order to explore the pharmacokinetic properties of MP. First, we investigated MP samples for the presence of a compound with DDC-like

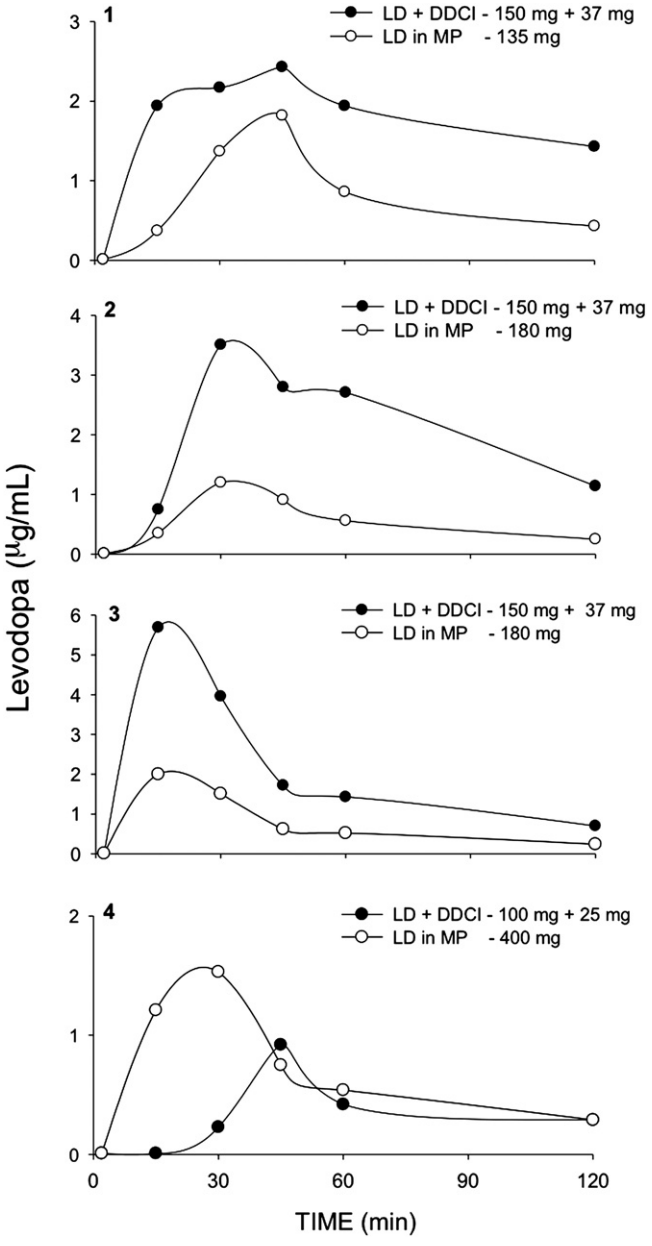

Fig. 2. Levodopa plasma pharmacokinetic measures.

activity, such as genistein and its precursor genistin, whose presence in MP has been postulated [7,16], but we did not find it. In second instance, we compared Levodopa AUC after intake of MP or standard Levodopa + DDCI, finding a 3.5 fold lower median ratio. Taken as a whole, the present findings do not support the presence of intrinsic DDCI-like activity. However, we cannot completely exclude a potential residual DDCI effect played by the last Levodopa + DDCI intake 12-h before the challenge. When Levodopa was administered at a 4-fold dose as MP powder in a patient with advanced PD, we found a similar improvement in motor symptoms along with longer ON duration and reduced dyskinesias (Table 2). Although limited by the single-case description, these clinical data are consistent with previous findings in human subjects [11,22]. Notably, our patient used MP powder from roasted MP seeds without any additional compound or pharmaceutical process, while PD subjects in the previous clinical study were administered a pharmaceutical product containing MP extract along with additives [11]. Using a 4-fold dose, the AUC was slightly greater (1.7-fold) with MP than with Levodopa + DDCI (Table 2, Fig. 2). Double-blind, randomized, controlled trials are required to assess the safety and efficacy of MP powder obtained from raw seeds in comparison to both Levodopa + DDCI and Levodopa without DDCI.

5. Conclusion

MP contains a mean of 5.3% Levodopa, albeit with substantial variability according to ecotype and preparation method. The lack of alkaloids indicates that MP is a safe source of Levodopa. If MP is safe and effective in the long-term, it may become a sustainable therapeutic alternative for patients with PD who live in tropical developing countries and who cannot afford pharmacological Levodopa therapy.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jns.2016.04.001.

Conflict of interest

None.

Acknowledgments

We wish to thank: the “Fondazione Grigioni per il morbo di Parkinson” and the “Prosolidar Foundation Onlus” (for financial support) (099-2013_GH), Jennifer S Hartwig, MD (for assistance in editing the manuscript) and Labanalysis Casanova Lonati (for the laboratory assessment).

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