Analysis of Levodopa Content in Commercial *Mucuna pruriens* Products Using High-Performance Liquid Chromatography with Fluorescence Detection

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Abstract

Objectives: *Mucuna pruriens* (MP) seeds contain levodopa (up to 2% by weight) and have been used in traditional Indian medicine to treat an illness named “Kampavata,” now understood to be Parkinson’s disease (PD). Studies have shown MP to be beneficial, and even superior, to levodopa alone in treating PD symptoms. Commercial products containing MP are readily available from online and retail sources to patients and physicians. Products often contain extracts of MP seeds, with significantly higher levodopa content than the seeds. However, MP products have limited regulatory controls with respect to quality and content of active ingredient. The aim of this study was to apply a quantitative method to determine levodopa content in readily available MP products that might be used by patients or in research studies.

Design: Levodopa present in six commercial MP products was quantified by solvent extraction followed by reversed-phase high-performance liquid chromatography (HPLC) coupled to fluorescence detection (FD). Certificates of analysis (COA) were obtained, from manufacturers of MP products, to assess the existence and implementation of specifications for levodopa content.

Results: HPLC-FD analysis revealed that the levodopa content of the six commercial MP products varied from 6% to 141% of individual label claims. No product contained levodopa within normal pharmacopeial limits of 90%–110% label claim. The maximum daily dose of levodopa delivered by the products varied from 14.4 to 720 mg/day. COAs were inconsistent in specifications for and verification of levodopa content.

Conclusions: The commercial products tested varied widely in levodopa content, sometimes deviating widely from the label claim. These deficiencies could impact efficacy and safety of MP products used by PD patients and compromise the results of scientific studies on MP products. The HPLC-FD method described in this study could be utilized by both manufacturers and scientific researchers to verify levodopa content of MP products.

Keywords: *Mucuna pruriens*, levodopa, HPLC, commercial products, analysis, Parkinson’s disease

Introduction

MUCUNA PRURIENS (L.) DC. (MP) is a legume native to India. As far back as 1500 BC, Ayurvedic medical texts discuss the use of MP seeds to treat a shaking palsy termed “Kampavata,” likely Parkinson’s disease (PD).¹ Kampavata describes a syndrome of tremors, rigidity, diminishing inclination to move, drooling, somnolence, and fixation of the

eyes.¹ A study published in 1937 demonstrated that MP contains levodopa (L-dihydroxyphenylalanine [L-DOPA]).² To date, levodopa remains the most effective treatment for motor symptoms of PD.

In animal studies, MP has been shown to be as effective as, and even superior to, levodopa alone in improving PD symptoms.^{3,4} In the 6-hydroxydopamine-induced rat model of PD, MP extract containing 2 mg of levodopa improved

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motor and sensory-motor deficits, whereas 6 mg of pure levodopa was required for a similar effect.³ MP was also more effective than an equivalent dose of levodopa in improving jaw movements induced by tacrine, an accepted model for Parkinsonian tremor.³ Interestingly, MP caused less drug-induced dyskinesia than synthetic levodopa.^{3,4} In addition to improving behavioral deficits, MP was shown to have neuroprotective and antioxidant effects in MPTP- and paraquat-induced mouse models of PD, respectively.^{5,6} In these mouse models, the non-levodopa components of MP appear to confer additional benefits in PD over those seen with levodopa alone.^{3,4}

In limited clinical studies, MP has again been shown to be effective in treating PD symptoms. Significant improvements have been seen in Hoehn and Yahr and United Parkinson's Disease Rating Scales I, II, and III following 12 weeks of MP treatment.⁷ A double-blinded crossover study of MP and synthetic carbidopa/levodopa showed a more rapid onset of effect and a longer "on" time with MP without a worsening of dyskinesias.⁸

In the United States, various commercial preparations of MP seeds are available through the internet or from retail outlets. As a result of the reported effectiveness of MP in treating PD symptoms, many patients in the United States are using these products.^{9,10} The products are classified as "dietary supplements," are not FDA approved for PD, and are relatively unregulated compared to conventional medicines.¹¹ Consequently, inconsistencies can occur in the level of active compounds, and appropriate analytical methods are required to address this issue.¹¹

In this study, a method was developed to quantify levodopa content in a set of MP products purchased online. This enabled an evaluation of the range of daily dose of levodopa delivered by these different products. Certificates of analysis (COA) issued by manufacturers of MP products were examined, when available, for evidence of in-house standards for levodopa content.

Materials and Methods

Materials

The following MP products were obtained from online sources: "Mucuna Pruriens" (Lot A1271130; Exp. November 2013; Herbal Powers), "Mucuna" (Batch no 20; Exp. July 2014; Herbsforever), "Mucuna" (Lot: 1012013; Exp. January 2014; Advance Physician Formulas), "DopaBean™" (Lot 151507; Use by December 2014; Solaray), "Mucuna Dopa" (FG-845; Best if used by December 2014; Source Naturals), and "Zandopa" (Batch No: BY009; March 2011; February 2013; Zandu). Reference standards of levodopa (3-(3, 4-dihydroxyphenyl)-L-alanine), and L-tyrosine (3-(4-hydroxyphenyl)-L-alanine) and ascorbic acid were obtained from Sigma-Aldrich. Ethanol, methanol (laboratory and high-performance liquid chromatography [HPLC] grade), hydrochloric acid, and formic acid were obtained from Fisher Scientific and phosphoric acid from JT Baker.

High-performance liquid chromatography

Reversed-phase HPLC was performed using an Econosil 5 μ m C18(2) 250 $\times$ 4.6 mm column, eluting at 1 mL/min with a methanol: 0.1% aqueous phosphoric acid gradient (% methanol 2, 2, 98, 98, 2, and 2, at 0, 10, 15, 20, 25, and 30 min, respectively), coupled to fluorescence detection (FD; excitation 282 nm and emission 630 nm). Levodopa and L-tyrosine eluted at 16.9 and 26.7 min, respectively (Fig. 1).

Selection of extraction solvent

To select the best extraction solvent from among those used in other MP studies, duplicate samples (100 mg) of Product 6 were extracted with one of four solvents (20 mL): ethanol:water 1:1 containing 0.1% ascorbic acid (EWA),¹² methanol:anhydrous formic acid 1:1 (MF),¹³ 0.1 N HCl,¹⁴ or 2% formic acid (FA).¹⁵ A preliminary study showed complete

HPLC conditions:

Column: Econosil 5 μ m C18(2) 250 $\times$ 4.6mm;
Gradient elution with methanol: 0.1% phosphoric acid in water;
flow rate 1ml/min. Injection volume: 10 μ l
Fluorescence detection: Excitation: 282nm; Emission: 630nm

FIG. 1. Levodopa is readily analyzed using reversed-phase HPLC coupled to fluorescence detection and with tyrosine as internal standard. HPLC, high-performance liquid chromatography.

extraction of levodopa from products using 20 mL MF. The amount of levodopa extracted by all four solvents was found, by HPLC, to be virtually identical. To examine stability, the extracts were stored at room temperature and reanalyzed after 24 and 96 h. Levodopa was most stable in solvent FA (only 6% deterioration at 96 h) and least stable in EWA (40% deterioration at 96 h). FA was therefore used in the quantitative study.

Calibration curve

Solutions with a range of levodopa concentrations (0–100 µg/mL) and a constant concentration of an internal standard, tyrosine (100 µg/mL) in FA, were analyzed by HPLC. A linear calibration curve was obtained in the range 0–100 µg/mL ($r^2 = 0.999$).

Analysis of levodopa content of six commercial MP products

Analysis of levodopa content was performed in February 2012, at which time all products were within their expiration date (see Materials section). For each of products 1–5, the powder contents of five randomly chosen capsules were combined and weighed. Product 6 was sold in free powder

form. Triplicate 50 mg portions of each product powder were extracted with internal standard solution (tyrosine 100 µg/mL in FA; 20 mL) and diluted further with internal standard solution, if necessary, to bring levodopa concentration within the linear range of the calibration curve. Extracts were analyzed by HPLC-FD. Levodopa in the extracts was quantified by comparing peak area ratios of levodopa/L-tyrosine for the extracts to a standard solution containing levodopa (50 µg/mL) in internal standard solution. Levodopa content in one serving was determined by calculation. The amount of levodopa delivered by the recommended maximum daily dose was also determined by calculation. The serving size and maximum daily dose were those suggested by the container label.

Certificates of analysis

COAs were provided by three manufacturers of MP products in response to a telephone enquiry.

Results

Levodopa content was found to vary from as little as 6% to 141% of the label claim (Table 1). None of the products was within normal pharmacopeial limits of $\pm 10\%$ of the

TABLE 1. COMPARISON OF EXPECTED LEVODOPA CONTENT FROM LABEL CLAIM WITH THE AMOUNT MEASURED BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-FLUORESCENCE DETECTION

Product number ^a	Label claim	Single dose (Serving size)	Levodopa CLAIMED mg/dose	Levodopa FOUND mg/dose (Mean% OF CLAIM $\pm$ SEM) ^b	Recommended maximum daily dose	Levodopa content in maximum daily dose of analyzed product (mg)
1	<i>Mucuna Pruriens</i> 60% L-DOPA: 100 mg	1 capsule	60	3.4–3.8 (6.0% $\pm$ 0.2%)	4 capsules	14.4
2	<i>Mucuna (Mucuna pruriens)</i> (Kaunch) seeds 25% L-DOPA. (135 mg per capsule)	1 capsule	33.8	10.2–13.4 (34.0% $\pm$ 3.0%)	3 capsules	34.5
3	Cowhage extract (standardized to contain 15% L-DOPA): (<i>Mucuna pruriens</i>) (seed): 200 mg	1 capsule	30	11.5–12.6 (40.0% $\pm$ 1.0%)	2 capsules	24
4	Velvet Bean (<i>Mucuna pruriens</i>) (seed extract) 333 mg; (Guaranteed 66 mg [20%] catecholamines, including 50 mg [15%] L-DOPA)	1 capsule	50.0	54–58 (111.0% $\pm$ 2.0%)	2 capsules	111
5	<i>Mucuna pruriens</i> seed extract 166 mg, yielding 100 mg L-Dopa	1 capsule	100	115–125 (120.0% $\pm$ 3.0%)	6 capsules	720
6	Each 7.5 g contains standardized processed seed powder of <i>Mucuna pruriens</i> Bak.6.525 g in a flavored base	“As directed by the physician”	250 ^c	333–374 (141.0% $\pm$ 3.0%)	2 scoops	705

^aSpecific identities of Products 1 to 6 may be requested from the corresponding author.

^bMeasured by HPLC-FD.

^cLabel for Product 6 does not state levodopa content, serving size, or recommended dosage, but a 7.5 g scoop is provided. Internet sites indicate 7.5 g has 250 mg levodopa. Two scoops of powder were used to determine daily dose of levodopa.

FD, fluorescence detection; HPLC, high-performance liquid chromatography; L-DOPA, L-dihydroxyphenylalanine; SEM, standard error of the mean.

stated amount. Products varied on recommended maximum daily dose of levodopa. In addition, analysis did not match the label claim. The maximum daily dose of levodopa that would actually be delivered by the six different MP products ranged from 14.4 to 720 mg/day (Table 1).

COAs were available for three brands of MP product. Only one COA corresponded to a brand tested by HPLC (Product 2), but was for a different batch from that analyzed. The COA showed a result of 21.2% levodopa despite the label claiming 25%, a discrepancy of >15%. In addition, no specifications were stated in the COA for levodopa content, suggesting that while levodopa was measured, there was no requirement for the batch to meet a value within 10% label claim. The COA for another brand had an assay result (62.4 mg) that was within 5% of the stated specification (60 mg) for levodopa. A third COA had neither a specification nor an assay result suggesting that levodopa was not routinely measured for that product. This demonstrates wide variation in the level of quality control applied by different manufacturers of MP products.

Discussion

This study utilized a simple solvent extraction protocol, followed by reversed-phase HPLC with FD, to accurately quantify levodopa content in commercial MP products available from online sources. A linear relationship ($r^2=0.999$) between levodopa concentration and levodopa peak area ratio to internal standard (tyrosine) was observed over the expected concentration range of our samples.

None of the measured levodopa contents for the six commercial MP products analyzed by HPLC matched the amount stated on the product label (Table 1). Product content ranged from as low as 6% to as high as 141% of the label claim; only Product 4 had a value close to the amount claimed. The possible reasons for discrepancies between the label claim and measured amount are varied. As demonstrated by the COAs obtained, manufacturers do not necessarily ensure the correct levels of levodopa as part of their quality control procedures, despite the claims made on product labels. Many dietary supplement manufacturers repackaging extracts prepared by a third party, and may not independently verify the levodopa content values provided by that supplier. Such verification is particularly important for natural products, where variability in components is expected, and has been demonstrated for natural levodopa levels in MP.^{16,17} Degradation of levodopa¹⁷ after manufacture may also explain low values as seen for Products 1–3. However, all products were analyzed within 6 months of being bought and at least a year before the earliest expiration date listed. One limitation of this study was the lack of assessment of multiple batches of a single product. The results are therefore batch specific and do not account for the possibility that other batches of that brand may have levodopa amounts closer to label claims.

Another finding in this study was that the daily levodopa dose delivered by suggested doses of different commercial products covered a wide range (Table 1). This variation reflects both the absence of standardized doses of MP and the observed differences between stated and actual levodopa content. Non-levodopa components may also vary between products, although this was not examined in this study. These discrepancies could severely impact the degree of relief

obtained for patients with PD and decrease the assurance with which doctors can support the use of MP products in PD. On the other hand, despite low levodopa content, PD patients may still derive some benefit from MP products due to the presence of non-levodopa constituents. For example, the studies described earlier demonstrated lower dyskinesias^{3,4} and greater on-time⁸ than with levodopa alone, as well as possible neuroprotective effects^{5,6} of MP.

Scientists performing research on MP should be aware of potential natural variations in levodopa levels, as well as inaccuracies in label claims of commercial MP products that they may wish to use in their research. Independent verification of levodopa content is recommended for such studies. The HPLC-FD method described in this study or other methods from the literature^{13,14,16,18–20} could be utilized for this purpose.

A popular reported method of measuring levodopa in MP extracts is high-performance thin layer chromatography (HPTLC).^{13,17,18} HPTLC has the advantage of requiring relatively unsophisticated equipment compared to HPLC and the ability to simultaneously evaluate multiple samples in parallel, on one plate. However, HPLC offers greater sensitivity, quantitative accuracy, and resolution of components compared to HPTLC. HPLC coupled to UV detection has also been utilized for measuring levodopa in MP.^{14,19} Our use of a FD coupled to HPLC in this method takes advantage of levodopa's unique fluorescence characteristics, and thus offers greater selectivity than the UV detection used in previously reported HPLC methods.^{14,19}

Conclusions

MP is a natural source of levodopa, with some scientific evidence for its ability to relieve PD symptoms. MP is a complex natural product with both levodopa and non-levodopa constituents, and there is evidence for a beneficial role in PD for both types of constituent. Given its potential benefit to PD patients, future studies on MP are warranted. With regard to evaluating the safety and effectiveness of MP in PD clinical trials, careful chemical profiling of product is necessary to ensure accurate dosing and reproducibility of outcomes. This study reports a standard methodology to evaluate levodopa content, which was applied to online commercial MP products. The results demonstrated significant differences between levodopa content and product label claims in the batches tested. The significance of this finding is that product deficiencies could impact efficacy and safety of MP products used by PD patients and compromise the validity of scientific research performed on MP products that have not been independently verified for levodopa content. Levodopa analysis should also be a first step in screening MP products for use in preclinical and clinical studies. This study underscores the importance of establishing independent testing of botanical products used in scientific research. This report also provides an analytical method (HPLC-FD) that could be of use for this purpose. The method could also be adopted by manufacturers to ensure correct and reproducible levodopa levels in MP products, thereby ensuring optimum results for patients.

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Author Disclosure Statement

No competing financial interests exist.

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