PHYTOTHERAPY RESEARCH, VOL. 11, 419–423 (1997)

Mucuna pruriens Proves More Effective than L-DOPA in Parkinson's Disease Animal Model

Ghazala Hussain and Bala V. Manyam*

Department of Neurology, Southern Illinois University School of Medicine, PO Box 19230, Springfield, Illinois, USA

Seeds of the *Mucuna pruriens* plant, now known to contain L-DOPA, have long been used for the treatment of Parkinson's dissease patients in ancient Eastern Indian ethnotherapeutics. Following validation of the intrastraital 6-OHDA injection with amphetamine in the parkinsonian rat model, the animals were fed synthetic L-DOPA (125 or 250 mg/kg) or *Mucuna pruriens* endocarp (MPE, 2.5 or 5.0 g/kg) mixed with rat chow ($n=6$, for each dose and drug). Controls received no drug. An additional dose of L-DOPA or MPE in the same doses plus carbidopa (50 mg/kg) were administered via gavage (controls received only carbidopa 50 mg/kg) 1 h prior to testing with rotometer. Contralateral rotation (to the side of the 6-OHDA lesion) (CLR) was recorded for 240 min as a measure of antiparkinsonian activity. Results indicated that dose for dose, MPE showed twice the antiparkinsonian activity compared with synthetic L-DOPA in inducing CLR in the parkinsonian animal model. This study suggests that MPE may contain unidentified antiparkinsonian compounds in addition to L-DOPA, or it may have adjuvants that enhance the efficacy of L-DOPA. © 1997 by John Wiley & Sons, Ltd.

Phytother. Res. **11**, 419–423, 1997
No. of Figures: 1. No. of Tables: 2. No. of References: 30.

Keywords: Mucuna pruriens; Parkinson's disease; Ayurveda; L-DOPA; 6-hydroxy-dopamine; rat.

INTRODUCTION

Parkinson's disease (synonym – paralysis agitans), a degenerative disease of the nervous system due to the progressive loss of nigrostriatal dopaminergic neurons and decrease in striatal dopamine, is characterized by the presence of tremor, muscular rigidity, poverty of movement, difficulty with balance and walking, depression and dementia. Most symptoms can be controlled through the use of drugs, but no single drug is completely effective. 3-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA), a dopamine precursor, either alone or in combination with an aromatic amino acid decarboxylase inhibitor (carbidopa, benserazide) are the most effective drugs for the treatment of Parkinson's disease. Additional drugs such as dopamine agonists, anticholinergics, amantadine or selegiline, are added as adjuvants. Hence, polypharmacy is not uncommon. All these drugs are available in the synthetic form. The use of multiple drugs to control the symptoms of a single disease is expensive (Harris *et al.*, 1991).

The exploration of ethnopharmacologic treatments may be a cost effective alternative, since naturally occurring compounds have been used in the treatment of Parkinson's disease. For example, the seeds of the datura plant have an anticholinergic effect and have been employed for several decades as an antiparkinsonian drug. The beans of the *Mucuna pruriens*, other members of the Mucuna family (Damodaran and Ramaswamy, 1937; Bell and Janzen, 1971; Daxenbichler *et al.*, 1971) and *Vicia faba* (Lattanzio *et al.*, 1982) contain levodopa, a major drug used in the treatment of Parkinson's disease. The DA agonist, bromocriptine, is extracted from the fungus — *Claviceps purpura* (ergot) (Parkes, 1977). *Mucuna pruriens* may also have DA agonist activity (Vaidya, R.A. *et al.*, 1978). Monoamine oxidase inhibitors are known to exist in plant sources such as *Banisteria Caapi* (Banisterine) (Sanchez-Ramos, 1991) and *Nicotiana tabacum*, the common tobacco plant (Norman *et al.*, 1982). Beans from *Mucuna pruriens* (Atmagupta, Sanskrit) have been described as a useful therapeutic agent in various diseases of the human nervous and reproductive systems in the ancient eastern Indian medical system known as *Ayurveda* (Nadkarni, 1908; Vaidya, N.D., 1925; Singhal *et al.*, 1979; Dutt, 1980). Indian scientists isolated L-DOPA from the beans of the *Mucuna pruriens* (Damodaran and Ramaswamy, 1937), but the role of L-DOPA in the treatment of Parkinson's disease was not known until 30 years later. Our studies have shown the L-DOPA content (dry weight) to be 4.02% in the whole *Mucuna pruriens* bean, 0.09% in the pericarp, and 5.28% in the endocarp (Mahajani *et al.*, 1996). Vaidya A.B., *et al.*, (1978) treated Parkinson's disease patients with a powder made from the beans of *Mucuna pruriens*. The results showed a decreased incidence of adverse effects compared with those treated with synthetic L-DOPA, which the patients were taking prior to ingesting the Mucuna powder. In a more recent clinical trial, utilizing a commercial product (HP-200) derived from *Mucuna pruriens* endocarp (MPE) a significant improvement of symptoms occurred in 60 patients with Parkinson's disease (HP-200 in Parkinson's Disease Study Group, 1995).

The objective of this study was to compare MPE with L-DOPA at two different dose levels utilizing the 6-hydroxy-dopamine (6-OHDA) induced parkinsonian rodent model to test our hypothesis that with the same dose of L-DOPA in the synthetic form or in the natural form, as it exists in *Mucuna pruriens* endocarp, the latter may be a

* Correspondence to: B. V. Manyam, Department of Neurology, Southern Illinois University School of Medicine, PO Box 19230, Springfield, Illinois, USA.

Contract grant sponsor: National Institutes of Health Office of Alternative Medicine.
Contract grant number: RFA 00-93-002.

CCC 0951–418X/97/060419–05 $17.50

Accepted 30 January 1997

more effective antiparkinsonian drug possibly due to the presence of additional unidentified antiparkinsonian compounds or adjuvants that enhance the efficacy of L-DOPA. The quantitative recording of rotational behaviour in the rats after 6-OHDA lesions of the nigrostraital dopamine system will be used for testing the antiparkinsonian effect of the two drugs (Ungerstedt and Arbuthnott, 1970).

METHODS AND MATERIALS

Male Sprague-Dawley rats (195–220 g) from Harlan Sprague-Dawley (Indianapolis, IN) were used for the study. Animals were housed, two per cage, at 25°±2°C on a 12 h light/dark cycle with free access to food and water. Animal procedures were conducted in strict accordance with *The Guide for the Care & Use of Laboratory Animals as adopted by National Institutes of Health.* All protocols were approved by the institutional animal care committee.

Sources for drugs. L-3,4-dihydroxyphenylalanine methyl ester, 6-OHDA hydrobromide, d-amphetamine sulphate, and apomorphine hydrochloride from Sigma Chemical Co., St. Louis, MO; *Mucuna pruriens* endocarp from Zandu Pharmaceutical Works, Bombay, India; carbidopa from Du Pont Pharma, Wilmington, DE, ketamine from Henry Schein, Port Washington, NY; xylazine from Miles Inc., Shawnee Mission, Kansas; and desimipramine from Sigma Chemicals, St. Louis, MO.

Surgical procedures. The rats (n=71) were pretreated with desimipramine (25 mg/kg *sc*) 45 min before surgery to prevent the high affinity uptake of 6-OHDA into noradrenaline neurons. Animals were then anaesthetized with an intraperitoneal injection of a cocktail of ketamine (40 mg/kg) and xylazine (5 mg/kg), and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with a bite-block 3.3 mm below the horizontal bar. A dental drill was used to make a single burr hole at the appropriate site on the right side of the skull. 6-OHDA [8 μg hypobromide salt dissolved in 4 μL of vehicle (sterile nitrogen bubbled 0.9% saline containing 0.02% ascorbic acid) stored at −70°C] injected at two separate sites into the corpus striatum as follows: the initial injection was made with the needle bevel directed rostrally at the following coordinates; AP+3.7 mm; ML-2.3 mm; DV-7.1 mm, and the second injection was made with the needle bevel directed laterally at the following coordinates: AP+3.7 mm; ML-1.9 mm; DV-7.4 mm, with respect to lambda and dura, based on Paxinos and Watson Stereotaxic Atlas (1986). 6-OHDA was infused at a rate of 1 μL/min through a Hamilton 10 μL syringe. After completion of each injection, the needle was left in place for 3 min in order to allow for diffusion of the toxin away from the needle tip. The incision site was cleaned with Betadine solution and the skin closed with wound clips.

Amphetamine treatment. The efficacy of the 6-OHDA lesion was tested with 2.5 mg/kg i.p. amphetamine 1 week after surgery and 5 mg/kg i.p. during weeks 2–4 using an automated rotometer for rotational behaviour (Ungerstedt and Arbuthnott, 1970; Schultz and Ungerstedt, 1978; Walsh and Silbergeld, 1979). Following administration of amphetamine, rats (n=66) were placed in plastic buckets and allowed to rotate. With the destruction of nigrostriatal dopaminergic neurons on the right side, amphetamine induced the animals to turn ipsilaterally. The number of complete rotations, ipsilateral and contralateral to the side of 6-OHDA lesion were recorded. Of the 66 animals lesioned, 40 completed seven or more ipsilateral rotations/min over a 90 min period in response to amphetamine, satisfying the criteria for adequate damage to the nigrostriatal dopaminergic neurons by 6-OHDA.

Method of recording rottaional behaviour in a rotometer. Rotations were measured using a 'rotometer' similar to that described earlier (Ungerstedt and Arbuthnott, 1970; Schultz and Ungerstedt, 1978; Walsh and Silbergeld, 1979) except that we used a software programme to enter data directly into the computer (Mac-Rotometer Processor, Maryland Scientific & Machining, Inc., Kensington, MD). A 'harness' consisting of a well fitting broad band was fastened around the chest of the animal just behind the forelimbs. On the dorsal surface of the rat, the band was hooked to the base of a male lure-lock fitting which connected the animal to a 0.4 mm steel wire having a female fitting. The wire transferred the movements of the animal via ball-bearing and cam to a microswitch that reacted to each full turn. Up to six animals were tested simultaneously, one in each bucket. Each full turn was registered on an electromechanical counter which was turned off, zeroed and turned on again by the Mac-Rotometer Processor. Each rat was kept in a cylindrical-shaped plastic bucket with the ball-bearing positioned at the geometrical centre of the bucket. This design restricted the movements of the animal to within the confines of the bucket keeping the wire connected to the machine from being stretched while allowing free ipsilateral rotation (ILR) to the side of 6-OHDA lesion, in this case the right side, and free contralateral rotation (CLR) to the side of 6-OHDA lesion. Turns per minute were plotted against time. Rats were monitored continuously during the experiments.

Sixty-one percent of the selectively lesioned rats met the criteria of <7 ILR/min during the initial 90 min in response to amphetamine injection (Perese *et al.*, 1989) and were included for testing the antiparkinsonian effect of MPE and L-DOPA.

L-DOPA and *Mucuna pruriens* endocarp treatment. Following 8 weeks of recovery and completion of amphetamine studies, 30 6-OHDA lesioned rats which met the criteria for ipsilateral rotation with emphetamine (7 rotations/min) were assigned to one of the following five drug treatment groups (n=6 per group): (1) control, received no drug; (2) L-DOPA (125 mgkg body wt.); (3) L-DOPA (250 mg/kg); (4) MPE (2.5 g/kg); (5) MPE (5 g/kg). Rats were fed *ad libitum* for 4 weeks with their respective doses mixed with rat chow (Purina Mills Inc., Richmond, IN). From weeks 2 to 4 of the treatment period, rats were orally treated either with their respective treatment drug dose (L-DOPA 125 mg/kg, L-DOPA 250 mg/kg, MPE 2.5 g/kg, MPE 5 g/kg) or their treatment drug dose plus carbidopa (50 mg/kg). Rats were tested (after 60 min of their oral administration) on the rotometer for 240 min for their rotational behaviour. When treatment drug dose plus carbidopa was given, rats were pretreated with carbidopa (50 mg/kg) and after 60 min treated with the combination drugs (treatment drug plus carbidopa).

Statistical analysis. Statistical analysis was done using Microsoft Excel 4.0 software. All values are reported as group mean±SEM. Significance was accepted for values of

$p<0.05$. The tests used were: two-tailed paired Student's *t*-test, chi-square test and one-way analysis of variance (ANOVAs).

RESULTS

Effect of amphetamine on rotational behaviour

Within the first 5 min of administration of amphetamine (2.5 and 5 mg/kg) ILR were observed. The mean ILR ranged from 3.95±0.58 turns/min on week 1 to 10.28±1.02 turns/min during week 4 after surgery. Details are given in Table 1. Statistical analysis by ANOVA revealed no significant differences in the number of ILR during weeks 2–4, while a high degree of significance ($p<0.0001$) was seen in the number of ILR when week 1 was compared with weeks 2–4. The difference between ILR and CLR was highly significant ($p<0.0001$) by *t*-test indicating that amphetamine produced a significant degree of ILR secondary to ipsilateral destruction of nigrostriatal dopaminergic neurons. After each successive week of rotational testing with amphetamine CLR decreased (Table 1). As the main effect of amphetamine was on ILR, the number of CLR was significantly small.

Antiparkinsonian effect of MPE and L-DOPA on 6-OHDA lesioned rats

With the addition of carbidopa (50 mg/kg), the CLR significantly increased between treatment groups (ANOVA, $p=0.037$) as shown in Table 2. Further, comparison of means via 95% confidence limits shows that MPE 5.0 g/kg (combined with carbidopa 50 mg/kg) differed significantly from control, L-DOPA 125 mg/kg and 250 mg/kg (combined with carbidopa), and MPE 2.5 g/kg (combined with carbidopa) in producing a higher number of CLR (Fig. 1). Despite adequate dosing no statistical significance in the number of CLR either by L-DOPA alone or MPE alone was seen (Table 2). A paired *t*-test was performed on rotational behaviour on each of the drug treatments to evaluate whether there was a significant difference between first and second trials (of the duplicate run). No significant difference was seen thus showing that the rats did not change their rotational behaviour over time.

Table 1. The rotational effect of amphetamine in 6-OHDA rat model of Parkinsonism

Week	No. of rats	Dose (mg/kg)	ILR (90 min)	CLR (90 min)
1	40	2.5	360.48±52.19[a]	21.50±6.13
2	40	5.0	999.90±85.32[a,b]	7.60±5.98
3	40	5.0	1032.00±88.47[a,b]	2.63±1.28
4	37	5.0	973.57±94.57[a,b]	2.05±1.21

All values mean±SE; ILR, ipsilateral rotation (to the side of 6-OHDA lesion); CLR, contralateral rotation (to the side of 6-OHDA lesion); [a]significant ($p<0.0001$) compared with CLR by *t*-test; [b]significant ($p=0.0001$) compared with week 1 by ANOVA.

DISCUSSION

Parkinson's disease has been attributed to the loss of DA from the caudate nucleus and dopamine cell bodies from the substantia nigra (Hornykiewicz, 1964). The caudate nucleus has been implicated in the control of motor function (Ungerstedt and Arbuthnott, 1970). An imbalance in DA neuronal activity between the two caudate nuclei reveals itself through prevalent asymmetry in the form of vigorous rotation towards the less active side. Unilateral injection of 6-OHDA through a stereotaxic approach could induce loss of DA cells with little damage to the non-catecholamine containing neuronal system creating an asymmetry in the DA content resulting in the animal turning to the side of the lesion. This turning can be measured using a 'rotometer' designed to measure the rotational speed or number of turns per minute over a long period of time. Amphetamine given to the 6-OHDA lesioned animals induced ipsilateral rotations to the side of lesion, as amphetamine is known to increase DA turnover mainly by releasing DA from the nerve terminals (Fuxe and Ungerstedt, 1970) and thus it increases the existing imbalance between the enervated and denervated caudate nucleus. L-DOPA and DA agonists produce the opposite effect wherein the animal will rotate contralateral to the side of lesion. Because of this selective behaviour of the animal, the intrastriatal 6-OHDA parkinsonian model is considered suitable for screening new potential antiparkinsonian agents (Kaakkola and Teravainen, 1990).

Following unilateral intrastriatal 6-OHDA injections amphetamine induced ILR within the first week. ILR following amphetamine is considered to be related to the differential release of DA at the caudate nucleus and putamen due to loss of DA terminals on the lesioned side (Zetterstrom *et al.*, 1986) and the loss of DA terminals could have occurred almost immediately following 6-OHDA injection. The early effect of amphetamine to validate successful lesioning by 6-OHDA has a practical application as the rat parkinsonian model can be ready sooner for further experiments.

Table 2. The rotational effect of L-DOPA and *Mucuna pruriens* endocarp with and without carbidopa in 6-OHDA rat model of Parkinsonism

Drug	Dose	With carbidopa 50 mg/kg CLR*[a] (3.5 h)	ILR*[b] (3.5 h)	Signif. (*t*-test)	Without carbidopa CLR*[c] (3.5 h)	ILR*[d] (3.5 h)	Signif. (*t*-test)
Water (control)	–	2.58±0.71	13.67±2.89	$p=0.002$	3.58±2.22	11.25±3.65	$p=0.015$
L-DOPA	125 mg/kg	76.33±58.13	0.33±0.25	$p=0.006$	7.75±2.70	14.00±3.44	$p=0.035$
L-DOPA	250 mg/kg	198.75±102.27	1.17±0.70	$p=0.009$	1.83±0.97	15.75±3.21	$p=0.003$
MPE	2.5 g/kg	239.69±150.61	6.00±2.34	$p=0.08$	6.17±2.02	17.58±3.58	$p=0.028$
MPE	5.0 g/kg	634.94±253.58	2.00±0.79	$p=0.01$	9.50±4.38	20.00±7.13	$p=0.116$

Rotation values mean±SE; rotation duration 3.5 h; CLR, contralateral rotation (to the side of 6-OHDA lesion); ILR, ipsilateral rotation (to the side of 6-OHDA lesion); MPE, *Mucuna pruriens* endocarp; *ANOVA MPE and L-DOPA compared with control ([a]$p=0.0370$; [b]$p=0.1187$; [c]$p=0.6380$; [d]$p=0.3112$).

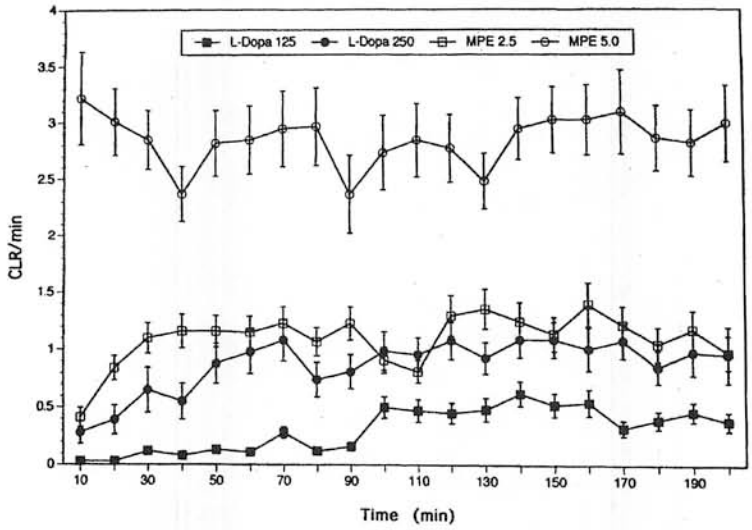

Figure 1. *Mucuna pruriens* endocarp (plus carbidopa) compared with L-DOPA (plus carbidopa) showing a significantly higher number of rotations contralateral to the side of the lesion in intrastriatal 6-OHDA rat model of parkinsonism. All animals received carbidopa (50 mg/kg) orally followed by a combination of carbidopa (50 mg/kg) and L-DOPA (125 mg/kg or 250 mg/kg) or carbidopa (50 mg/kg) and *Mucuna pruriens* endocarp (2.5 g/kg or 5.0 g/kg). Control animals received carbidopa only (not shown due to very low no. of rotations). All values mean±SE. CLR, contralateral rotations (to the side of 6-OHDA lesion). *Mucuna pruriens* endocarp (5.0 g/kg) with carbidopa (50 mg/kg) was significant (p=0.037) by ANOVA.

A large standard error was noted in CLR (Fig. 1) indicating variability in response by individual rats to the antiparkinsonian effect of L-DOPA and *Mucuna pruriens* endocarp despite the fact that all animals had adequate degeneration (90%) of nigrostriatal dopaminergic neurons by responding to amphetamine-induced circling behaviour. This may suggest that the individual variation was due to the behavioural nature of the study and a larger number of animals need to be used in future experiments. We did not undertake histopathological confirmation of the degree of tissue damage, but instead we will be subjecting the lesioned tissue to neurochemical analysis which will be reported separately. Further, being placed in the rotometer buckets for 240 min with the ability to move around, the rats would not stand still and rotated to a maximum of 20±7.13, in groups treated without carbidopa for both CLR and ILR, and for the group treated with carbidopa for ILR. But, a significant number of rotations in high double digits or triple digits occurred only in the group treated with carbidopa for CLR which is representative of antiparkinsonian activity. Nevertheless, the experiments provided information that L-DOPA, even at a dose of 500 mg/kg, may not produce adequate response of contralateral rotations to the side of the lesion unless carbidopa is added facilitating better the availability of L-DOPA in the central nervous system. Such an effect appears to be due to species variation. For example, the reported oral LD_{50} of L-DOPA in rats is 4000 mg/kg, in mice 3650 mg/kg and in rabbits 609 mg/kg (Budavari, 1989). In humans, the dose of synthetic L-DOPA needed to produce a clinical improvement in parkinsonian signs is 2 to 3 g/day in divided doses of 20% to 25% of that dose when combined with an aromatic L-amino acid decarboxylase inhibitor (carbidopa, benserazide). A dose of 200 mg/kg of MPE is more than adequate to produce similar effects in patients with Parkinson's disease (HP-200 IN Parkinson's Disease Study Group, 1995). The high degree of individual variation in the number of CLR under identical experimental conditions and identical dosage of drugs and the difference in species response to various drugs illustrates the limitations of animal experiments to verify human disease treatments. The 6-OHDA lesioned rat is a pharmacological model of Parkinsonism and not a true Parkinson's disease model as the aetiology of this neurodegenerative disease remains unknown.

The usual method in drug development is to screen a large number of drugs, or plant extracts for 'lead compounds' test in animal models for therapeutic activity, and then, if effective, conduct acute and chronic toxicological studies prior to conducting human clinical trials. This is an expensive and time consuming approach. For example, in 1981 the cost was $54 million and the time involved was 10 years (Wardell and Shek, 1983). This expensive approach may not be applicable in evaluating drugs from traditional systems of medicine such as *Ayurveda*, North American Indian Medicine, Chinese Medicine, etc. In these systems of medicine, an extract of the whole plant or part of a plant such as roots, leaves, seeds etc, are used. In addition to the active ingredients, the product may contain hundreds of other bioactive compounds which may act as either adjuvant or antidotes for some of the adverse effects produced by the lead compounds, or may aid metabolism or absorption. As an example, reserpine, the main alkaloid of *Rauwolfia serpentina*, administered for the treatment of depression, causes extrapyramidal adverse effects such as parkinsonism and tardive dyskinesia (Chase, 1972). The crude extract of the root of *Rawolfia serpentina*, which contains reserpine and a host of other unidentified alkaloids, has not been reported to cause extrapyramidal adverse effects (Manyam, 1990). MPE has a pH of 6.3 possibly due to the presence of ascorbic acid (0.12%, dry wt.) (Manyam *et al.*, unpublished data, 1994). It is well known that the solubility of L-DOPA requires an acidic pH. Despite these recently discovered facts, and the use of plant based products over centuries, the therapeutic efficacy and safety of a product developed from

ethnopharmacology cannot be taken for granted and must be reevaluated by subjecting it to rigorous testing similar to that for synthetic compounds prior to widespread use in humans. Data included in this paper confirm the efficacy of MPE as an antiparkinsonian agent in the intrastriatal 6-OHDA parkinsonian animal model. It also shows that MPE is more effective than its synthetic counterpart (Fig. 1). Our previous study of MPE has shown a pharmacokinetic effect similar to the combination of levodopa and carbidopa (Mahajani *et al.*, 1996). MPE has no significant acute or chronic toxic effect in rats and rabbits at four different doses (Manyam *et al.*, 1996, unpublished data), and a phase II clinical trial in humans has shown the product to be highly effective in patients with Parkinson's disease (HP-200 in Parkinson's Disease Study Group, 1995). These studies confirm our hypothesis that MPE may contain more than one antiparkinsonian compound in addition to L-DOPA or it may have adjuvants that enhance the efficacy of L-DOPA.

Acknowledgements

Supported by the National Institutes of Health Office of Alternative Medicine (Grant no. RFA 00–93–002). We thank K. M. Parikh, Zandu Pharmaceutical Works, for *Mucuna pruriens* endocarp; Du Pont Pharma for carbidopa; Shari B. Randall for technical assistance; Tracy A. Schatteman for assistance with data analysis; and Robert J. Plunkett for advice on stereotaxic procedure.

REFERENCES

Bell, E. A., and Janzen, D. H. (1971). Medical and ecological considerations of L-dopa and 5-HTP in seeds. *Nature* **229**, 136–137.

Budavari, S. ed. (1989). *The Merck Index*, 11th edn, pp. 860, Merck Res. Labs., Rathway, NJ.

Chase, T. N. (1972). Drug-induced extrapyramidal disorders. In *Proceedings of the Association for Research in Nervous and Mental Disorders*, pp. 448–466. Williams & Wilkins, Baltimore.

Damodaran, M., and Ramaswamy, R. (1937). Isolation of L-dopa from the seeds of *Mucuna pruriens*. *Biochemistry* **31**, 2149–2151.

Daxenbichler, M. E., VanEtten, C. H., Hallinan, E. A. and Earle, F. R. (1971). Seeds as sources of L-dopa. *J. Med. Chem.* **41**, 463–465.

Dutt, V. C. (1980). *Materia Medica of the Hindus* (Krishnades Sanskrit Studies Vol II), pp. 148–149. Chowhamba Saraswalibhawan, Varanasi.

Fuxe, K., and Ungerstedt, U. (1970). Histochemical, biochemical and functional studies on central monoamine neurons after acute and chronic amphetamine administration. In, *Amphetamines and Related Compounds*, ed. by E. Costa and S. Garattini, pp. 257–288. Raven, New York.

Harris, R. B., Hughes, L. F., Elble, R. J. and Manyam, B. V. (1991). Parkinson's disease: factors of importance in rehabilitation. *Tenth International Symposium on Parkinson's disease. Tokyo, Japan, 27–30 October.*

Hornykiewicz, O. (1964). Zur Frage des Verlaug's domaminerger Neurone im Gehirn des Menschen. *Wien. Klin. Wschr.* **767**, 834–835.

HP-200 in Parkinson's Disease Study Group (Manyam, B. V., Senior Author). (1995). HP-200: An 'Alternative Medicine' Treatment for Parkinson's disease. *J. Alternat. Comp. Med.* **1**, 249–255.

Kaakkola, S., and Teravainen, H. (1990). Animal models of Parkinsonism. *Pharmacol. Toxicol.* **67**, 95–100.

Lattanzio, V., Bianco, V. V. and Lafiandra, D. (1982). High-performance reversed-phase liquid chromatography (HPLC) of favism-inducing factors in *Vicia faba* L. *Experientia* **38**, 789–790.

Mahajani, S. S., Doshi, V. J., Parikh, K. M., and Manyam, B. V. (1996). Bioavailability of L-dopa from HP-200 — a formulation of seed powder of *Mucuna pruriens*. A pharmacokinetic and pharmacodynamic study. *Phytother. Res.* **10**, 254–256.

Manyam, B. V. (1990). Paralysis agitans and levodopa in "Ayurveda," ancient Indian medical treatise. *Mov. Disord.* **5**, 47–48.

Nadkarni, K. M. (1908). *Indian Plants and Drugs*, p. 242. Norton & Co., Madras.

Norman, T. R., Chamberlain, K. G., French, M. A., and Burrows, G. D. (1982). Platelet monoamine oxidase activity and cigarette smoking. *J. Affect. Dis.* **4**, 73–77.

Parkes, D. (1977). Bromocriptine. *Adv. Drug Res.* **12**, 248–331.

Paxinos, G., and Watson, C. (1986). *The Rat Brain in Stereotaxic Coordinates*, 2nd edn. Academic Press Ltd., London.

Perese, D. A., Ulman, J., Viola, J., Ewing, S. E., and Bankiewicz, K. S. (1989). A 6-hydroxydopamine-induced selective parkinsonian rat model. *Brain Res.* **494**, 285–293.

Sanchez-Ramos, J. R. (1991). Banisterine and Parkinson's disease. *Clin. Neuropharmacol.* **14**, 391–402.

Schultz, W., and Ungerstedt, U. (1978). Straital cell supersensitivity to apomorphine in dopamine-lesioned rats correlated to behaviour. *Neuropharmacol.* **17**, 349–353.

Singhal, G. D., Singh, R. H., and Shukla, K. P. (1979). Non-operative considerations in ancient Indian surgery (based on Susruta Samhita, Cikitsa-Sthana, chapters 24–40). Singhal Publications, Varanasi.

Ungerstedt, U., and Arbuthnott, G. W. (1970). Quantitative recording of rotational behavior in rats after 6-hydroxydopamine lesions of the nigrostriatal dopamine system. *Brain Res.* **24**, 485–493.

Ungerstedt, U., Avema, A. D., Ljundberg, T., and Runge, C. (1973). Animal models of Parkinsonism. *Adv. Neurol.* **3**, 257–271.

Vaidya, A. B., Rajogopalan, T. G., Mankodi, N. A. *et al.* (1978). Treatment of Parkinson's disease with the Cowhage plant — *Mucuna pruriens* bark. *Neurology India* **26**, 171–176.

Vaidya, N. D. (1925). *A Treatise on Experienced Remedies*, p. 100. Vaidya-Kalapataru, Ahmeabad.

Vaidya, R. A., Sheth, A. R., Aloorkar, S. D. *et al.* (1978). The inhibitory effect of the Cowhage plant — *Mucuna pruriens* — and L-Dopa on chlorpromazine-induced hyperprolactinaemia in man. *Neurology India* **26**, 177–182.

Walsh, M. J. and Silbergeld, E. K. (1979). Rat rotation monitoring for pharmacology research. *Pharma. Biochem. Behav.* **10**, 433–436.

Wardell, W. M., and Shek, L. E. (1983). Is pharmaceutical innovation declining? Interpreting measures of pharmaceutical innovation and regulatory impact in the USA 1950–1980. *Rational Drug Ther.* **17**, 1.

Zetterstrom, T., Herrara-Marschitz, M., and Ungerstedt, U. (1986). Simultaneous measurement of dopamine release and rotational behavior in 6-hydroxydopamine denervated rats using intracerebral dialysis. *Brain Res.* **376**, 1–7.