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Therapeutic and adverse effects of chronic oral intake of *Mucuna pruriens* seed extracts or L-DOPA methyl ester in 6-hydroxydopamine lesioned rats

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Introduction

Levodopa (L-DOPA, L-3,4-dihydroxyphenylalanine) is the most effective symptomatic agent in the treatment of Parkinson's Disease (PD) as a dopamine replacement therapy. Chronic use of L-DOPA is, however, known to induce dyskinesias (Abnormal Involuntary Movements in rats (AIMs)) (Jenner 2008).
Seeds of the legume *Mucuna pruriens* (MP) are a rich source of L-DOPA and preparations of MP seeds have long been used to treat PD in the traditional Ayurvedic Indian Medicine system (Singhal et al. 2003). It has been suggested that MP preparations induce less motor complications in PD patients than synthetic L-DOPA (Katzenschlager et al 2004). Methanol extraction of MP seeds (MP-MeOH) yields large amounts of L-DOPA (15-20%), whereas hexane extracts (MP-hex) are rich in lipophilic compounds like coenzyme Q_{10}.

Aim of study

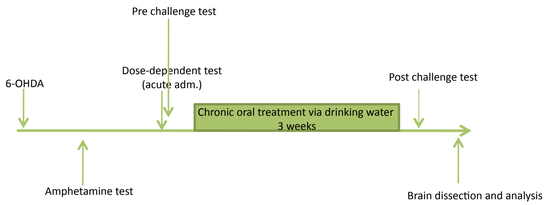
1. To investigate, in an animal model for PD, the acute, dose-dependent therapeutic and adverse effects of L-DOPA methyl ester (LDME) and MP-MeOH after intraperitoneal (ip) injections.
2. To monitor L-DOPA plasma levels in rats receiving LDME or MP-MeOH extract via the drinking water.
3. To study the development of adverse effect after chronic oral administration of MP-MeOH, MP-MeOH combined with ip injections of MP-hex or LDME preparations.

Method and materials

Animal model*:* unilaterally 6-hydroxydopamine (6-OHDA) lesioned male Sprague Dawley rats with > 98% striatal DA-depletion (left medial forebrain bundle, 8 µg 6-OHDA free base/2 µl 0.1% ascorbic acid)
Monitoring therapeutic and adverse effects: Cylinder test (therapeutic effects) and AIMs rating scale (adverse effects (dyskinesias)) (Lundblad et al 2002).
Drug preparations: LDME or MP-MeOH solubilized in ascorbic acid solutions together with benserazide (15 mg benserazide/kg, for ip administration or 1 mg benserazide/ml for oral administration). Solutions were normalized to specific L-DOPA concentrations. MP hexane extract was administered daily, 250 mg/kg, ip for 3 weeks.
Blood sampling and DOPA assay: Via permanent catheter (a. femoralis). DOPA was extracted with perchloric acid (PCA) and determined by HPLC with electrochemical detection.

Protocol

Acute dose-dependent tests: ip administration (LDME and MP-MeOH), (3, 6, 9 mg/kg)
Pre- and post challenge tests: ip administration (LDME), (6 mg/kg)
Chronic oral treatment: 3 weeks of oral administration via drinking water (LDME, MP-MeOH, MP-MeOH+hexan and control), (0.5-2.0 mg DOPA/ml), (MP-hex 250 mg/kg, ip)



Results

Figure 1
(A) Acute dose-dependent AIMs score after ip administration of MP-MeOH or LDME preparations (60 min post injection). Results are shown as mean ± SEM (LDME group: n=13-15; MP-MeOH group: n=13-16).
*** p<0.001, ANOVA with Bonferroni post-test
(B) Acute dose-dependent test of therapeutic effects after ip administration of MP-MeOH or LDME preparations (60 min post injection). Results are shown as mean ± SEM (BL=baseline groups: n=15-16; LDME group n=4-12, except LDME 9 mg/kg: no observations; MP-MeOH group: n=6-14, except MP-MeOH 9 mg/kg: n=2. Unequal number of observations is due to dyskinetic animals.
** p<0.01, ANOVA with Bonferroni post-test

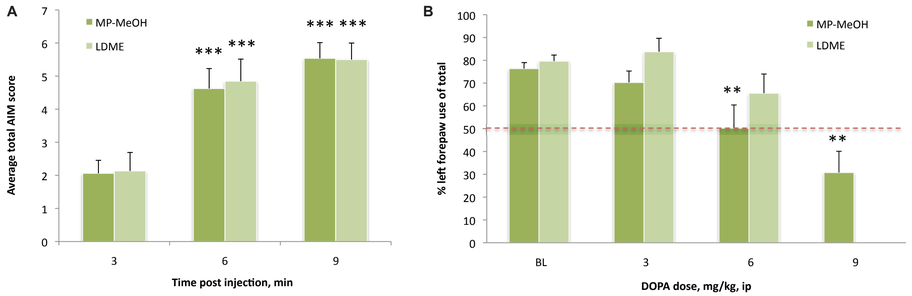


Figure 2
(A) Acute observed AIMs after ip administration of LDME (6 mg/kg) in different treatment groups, pre- and post chronic oral treatment via the drinking water. Results are shown as mean ± SEM. For all groups n=4.
(B) Therapeutic effects at different DOPA doses administered via the drinking water. Results are shown as mean ± SEM (n=6-14).
** p<0.01, ANOVA with Bonferroni post-test

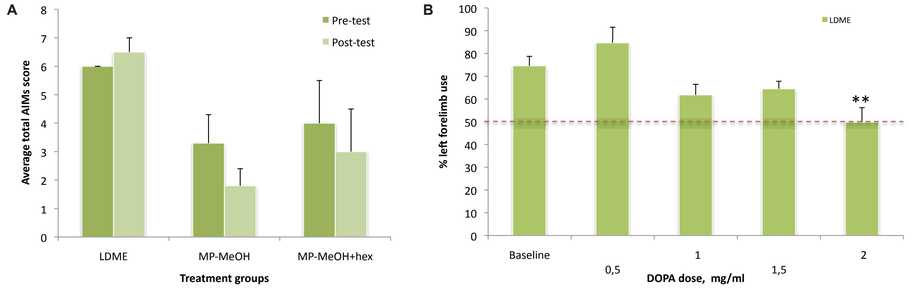
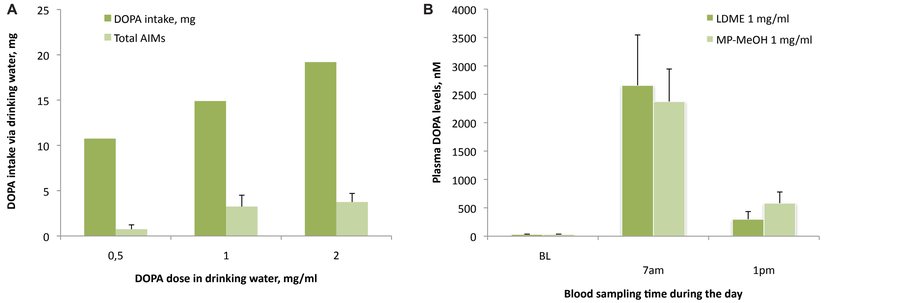


Figure 3
(A) DOPA intake (mg) via drinking water and observed average total AIMs score in LDME-treated rats. Results are shown as the mean DOPA intake in one home cage with 4 rats, measured by weight and mean total AIMs score ± SEM (n=4).
* p<0.05, r^2=0.337, linear regression (Goodness of Fit).
(B) Plasma DOPA levels after oral administration of LDME or MP-MeOH via the drinking water. Blood sampling was performed at various time points during the day. Results are shown as mean ± SEM. n=4-9, except LDME 1 mg/ml (7am): n=18



Discussion and conclusions

Acute dose dependent tests using LDME or MP-MeOH preparations (ip adm.), showed significant higher AIMs scores with increasing dose (***, p<0.001), with no difference between LDME and MP-MeOH preparations. AIMs were observed even at low dose (3 mg/kg) in both groups. The therapeutic effect was achieved with 6 mg DOPA/kg for MP-MeOH treated rats, but in LDME-treated rats this dose caused severe AIMs interfering with normal forelimb use.

Oral treatment via the drinking water caused large variations in plasma DOPA levels between morning and afternoon, but similar average plasma levels in LDME- and MP-MeOH-treated rats. A weak relation (*, p<0.05, r^2=0.337) was observed between the average amount of DOPA intake and the averaged total AIMs score.
Chronic oral treatment with LDME, MP-MeOH or MP-MeOH+hexane showed no significant differences in AIMs scores following a LDME challenge test (ip). However within treatment groups, pre- and post challenge tests did not show significant differences either, in contrast to chronic ip administration.

Figure 4
Seeds from *Mucuna pruriens*, the plants, pods and flowers

Acknowledgement

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References

Jenner, P. (2008) Nat Rev. Neurosci. 9, 665-677
Singhal, B., Lalkaka, J. and Sankhla, C. (2003) Parkinsonism Relat. Disord. 9 Suppl. 2, S105-109
Katzenschlager R., Evans A., Manson A., Patsalos P. N., Ratnaraj N., Watt H., Timmermann L., Van der Giessen R. and Lees A. J. (2004) *J Neurol Neurosurg Psychiatry* 75, 1672-1677
Lundblad, M., Anderson, M., Winkler, C., Kirik, D., Wierup, N. and Cenci, M.A. (2002) Eur J Neurosci. 15, 120-132