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## Effects of Five Ayurvedic Herbs on Locomotor Behaviour in a *Drosophila melanogaster* Parkinson's Disease Model

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### Abstract

Current conventional treatments for Parkinson's disease (PD) are aimed at symptom management, as there is currently no known cure or treatment that can slow down its progression. Ayurveda, the ancient medical system of India, uses a combination of herbs to combat the disease. Herbs commonly used for this purpose are Zandopa (containing *Mucuna pruriens*), *Withania somnifera*, *Centella asiatica*, *Sida cordifolia* and *Bacopa monnieri*. In this study, these herbs were tested for their potential ability to improve climbing ability of a fruit fly (*Drosophila melanogaster*) PD model based on loss of function of phosphatase and tensin-induced putative kinase 1 (PINK1). Fruit flies were cultured on food containing individual herbs or herbal formulations, a combination of all five herbs, levodopa (positive control) or no treatment (negative control). Tests were performed in both PINK1 mutant flies and healthy wild-type (WT) flies. A significant improvement in climbing ability was observed in flies treated with *B. monnieri* compared with untreated PINK1 mutant flies. However, a significant decrease in climbing ability was observed in WT flies for the same herb. *Centella asiatica* also significantly decreased climbing ability in WT flies. No significant effects were observed with any of the other herbs in either PINK1 or WT flies compared with untreated flies.

### Keywords

Ayurveda; climbing ability; *Drosophila melanogaster*; Parkinson's disease; PINK1

## INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder, only preceded by Alzheimer's disease in prevalence (Nass and Przedborski, 2008;

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### Conflict of Interest

The authors have declared that there is no conflict of interest.

Polymeropoulos *et al.*, 1996). About six million people are estimated to suffer from the disease worldwide, with some predictions estimating a two-fold increase over 25 years (Nass and Przedborski, 2008). Today, there is no known cure, nor is there any therapeutic intervention scientifically shown to slow its progression (Nass and Przedborski, 2008; Rascol *et al.*, 2011). The main symptoms of PD are motoric in nature: tremor-at-rest, bradykinesia, rigidity, loss of postural reflexes, flexed posture and the freezing phenomenon (Fahn, 2008; Jankovic, 2008). Patients often also suffer from non-motor symptoms, including depression, dementia and disturbed sleep (Wilkinson and Lennox, 2005). The main brain degeneration is selective loss of dopaminergic neurons of the *substantia nigra* (SN), especially in the *pars compacta* of the SN (Wilkinson and Lennox, 2005; Dickson, 2008). This causes a decrease in the amount of dopamine available in this part of the brain (Wilkinson, 2005). However, not only the SN is affected, but also many other brain areas are known to be involved in the neuropathology of PD, including parts of the limbic area and neocortex, the diencephalon and multiple brain stem areas not associated with motor control and the peripheral autonomic nervous system (Rascol *et al.*, 2011).

Neurons that degenerate in PD accumulate typical cytoplasmic inclusions containing mainly  $\alpha$ -synuclein and ubiquitin called Lewy bodies (LBs) (Dickson, 2008). LBs are not specific for PD but can also occur in other clinical syndromes, such as dementia, psychosis and dysautonomia (Dickson, 2008). LBs are partly caused by protein misfolding, and the presence of these aggregates is known as LB pathology (Braak and Tredici, 2008).

All current therapies are aimed at symptom management. Medication is often the main aspect of therapy, and its main focus lies in correcting the shortage of dopamine (Fahn, 2008). The current 'gold standard' drug is levodopa (Brooks, 2008). Another treatment option is dopamine agonists (DAs), which have longer plasma elimination half-lives than levodopa (Rascol *et al.*, 2011). DAs (e.g. bromocriptine, pergolide, lisuride, cabergoline, apomorphine, ropinirole and pramipexole) are able to reduce 'off' time and often allow lowering of levodopa dosage, reducing dyskinesia (Fahn, 2008; Rascol *et al.*, 2011). However, there is a greater risk of certain adverse drug reactions compared with treatment with levodopa alone, including abnormal daytime somnolence, ankle and leg oedema, and impulse control disorders such as hypersexuality and compulsive gambling, shopping and eating (Fahn, 2008; Rascol *et al.*, 2011). DAs are also more likely to cause hallucinations, confusion and psychosis, especially in older patients, and it is therefore safer to use levodopa in patients over the age of 70 years (Fahn, 2008).

A different type of dopaminergic treatment involves amantadine. Amantadine works in several ways: it has antimuscarinic effects, can activate release of dopamine from nerve terminals, block dopamine uptake into nerve terminals and block glutamate *N*-methyl-D-aspartate (NMDA) receptors (Fahn, 2008). It is a weak non-competitive NMDA antagonist, but its mechanism of action remains poorly understood (Rascol *et al.*, 2011). Amantadine is the only known antidyskinetic agent and can reduce the severity of levodopa-induced dyskinesia. Unfortunately, its effect tends to lessen over time (Fahn, 2008). Moreover, it can induce visual hallucinations, confusion, livedo reticularis and ankle oedema (Fahn, 2008). Other antimuscarinic agents are sometimes also used, although these are much less effective than dopaminergics (Fahn, 2008).

Ethnopharmacology, the study of the use of medicinal plants by different ethnic groups, may provide important clues for new treatments. Ayurveda, the Indian traditional system of medicine, uses a combination of herbs to treat PD, alongside treatments not involving medication. The herbs that are classically used for this are *Withania somnifera*, *Bacopa monnieri*, *Centella asiatica* and *Sida cordifolia* (Pioreschi, 1996; Chopra, 2003; Mishra, 2003, Williamson, 2002, Katzenschlager *et al.*, 2004). Additionally, *M. pruriens* seeds contain significant amounts of levodopa: the presence of  $29.8 \pm 1.2$  mg/g of dried tissue was reported by Nagashayana *et al.* (2000).

We sought to explore the therapeutic potential of these herbs to alleviate motor symptoms in PD. Our initial strategy was to first test whether these herbs may improve locomotion in a simple animal model of PD, the fruit fly (*Drosophila melanogaster*). *Drosophila melanogaster* has been extensively used to model PD (Whitworth, 2011) and provides a time-effective and cost-effective screening tool to evaluate the efficacy of potential therapeutics (Pandey and Nichols, 2011) Here, we used *D. melanogaster PINK1* mutants, which show several phenotypes characteristic of PD, including dopamine neuronal degeneration and locomotor defects (Park *et al.*, 2006; Muñoz-Soriano and Paricio, 2011). Positive results with this simple model system would support the investment of further work in additional pre-clinical models.

## MATERIALS AND METHODS

### *Drosophila melanogaster* stocks and culturing conditions

The PINK1 ( $w^-$  PINK1<sup>B9</sup>/FM7.GFP<sup>w+</sup>) flies were kindly provided by Dr Alex Whitworth (University of Sheffield), whereas the wild type (WT) flies were provided by Dr Barry Brogan (Newcastle University). Flies were kept in an incubator with a 12 h day–night cycle at 25 °C. Fly food was prepared by mixing Applied Scientific Jazz-Mix *Drosophila* Food (Fisher Scientific, Loughborough, UK) with the appropriate amount of water, bringing this to a boil while stirring, and stirring at low heat for another 10 min. The fly food was then poured into fly bottles (250 mL, Scientific Laboratory Supplies) and put into a freezer until it became solid, and the inside of the bottle was wiped clean with a paper towel before closing off the bottle with cotton wool.

### Chemicals

Levodopa (L-DOPA, 3,4-dihydroxy-L-phenylalanine) and vitamin C powder (L-ascorbic acid) were purchased from Sigma-Aldrich (Dorset, UK).

### Herbs

*Mucuna pruriens* powder was obtained from Zandu Pharmaceutical Works Ltd. (part of Emami Ltd., Kolkata, India) in the form of Zandopa, previously known as HP-200. Each 7.5 g of Zandopa contained 6.525 g of *M. pruriens* standardised processed seed powder in a flavoured base of which the composition is unknown. Organic *B. monnieri* powder, made from the aerial parts, was obtained from Pukka Herbs (Bristol, UK). *Withania somnifera* root was obtained from G Baldwin & Co (London, UK). *Centella asiatica* aerial parts from Proline Botanicals and *S. cordifolia* roots from Pukka Herbs (Bristol, UK) were kindly

gifted by Professor Elizabeth Williamson, University of Reading (UK). All dried herbs were ground to a powder using a coffee mill.

## Dosage

The equations by Hong *et al.* (2011) were used to convert dosages used in human studies to effective and non-toxic doses that could be used in the *Drosophila* experiments (Table 1). These dosages were not necessarily used to treat PD or have an effect on cognitive function. No studies investigating the effects of *S. cordifolia* on its own in humans were found; therefore, some manufacturers' recommendations were adopted for *S. cordifolia*.

To prevent oxidation of L-DOPA, 20.8 mg ascorbic acid was added to flasks containing L-DOPA, similar to the amount used in a study by Pendleton *et al.* (2002). However, ascorbic acid on its own may also affect the climbing ability of PINK1 flies (Khan *et al.*, 2012); therefore, flasks to which only ascorbic acid was added were used as an additional control. The negative control contained no herbs or chemicals.

## Treatment protocol

Food containing treatment was made by mixing the powdered herbs or the chemicals (Table 2). Depending on whether the herbs were roots, aerial parts or seeds, the herbs were added at different points during the process of preparing the food. Roots (*W. somnifera* and *S. cordifolia*) were added to the water along with the dry food mixture, before bringing it to a boil. Aerial parts (*B. monnieri* and *C. asiatica*) were added once the water was boiling; seeds (Zandopa), levodopa and ascorbic acid were added once the mixture was stirred for 10 min and had cooled down sufficiently. Each time, flies were allowed to lay eggs on food for 2 days before being transferred to fresh food. After 6 days, the flies were transferred to regular food and redistributed onto treatments the next day.

## Climbing assay

The severity of the symptoms in *PINK1* mutants was measured by testing their climbing ability. Fourteen days after the flies were originally placed on food with treatment, their progeny were tested using a climbing test adapted from Pendleton *et al.* (2002). Depending on the number of flies available, between three and 15 male flies were placed in a 100-mL measuring cylinder and allowed 10 min to acclimatise. The flies were then gently tapped down and allowed to climb up for about 15–20 s and then tapped down again. This was recorded and repeated two more times. At the third time, the number of flies passing the 100mL (16.5 cm) mark (escaped) within the first 10 s was recorded. The percentages of flies that escaped were then calculated.

## Data analysis

Minitab 16.1.0 (Minitab, Inc., (2009), Minitab Statistical Software, Release 16 for Windows, State College Pennsylvania) was used to check normality of data distribution for both WT and PINK1 flies. An adjusted two-way analysis of variance (ANOVA) with Bonferroni post-tests was performed using GraphPad Prism, version 5.03 for Windows (GraphPad Software, San Diego California USA, [www.graphpad.com](http://www.graphpad.com)). As the design was unbalanced (depending on availability of the flies), multiple regressions were performed three times, and each time,

the data were presented in a different order. Only the sums of squares for the factor entered last into the multiple regressions, called type III sums of squares, were displayed (GraphPad Software Inc.; 2013).

## RESULTS

Figure 1 shows the average percentage of flies that escaped for each treatment per fly type with the standard errors. The results of the two-way ANOVA are shown in Table 3. As can be seen from the table, there was a highly significant difference in percentage of escaped flies between fly types ( $p < 0.0001$ ), the treatments had a significant effect ( $p = 0.0157$ ) and there was a significant interaction between fly type and treatment ( $p = 0.0005$ ).

The PINK1 flies responded differently to the various treatments compared with WT flies. This can be seen, for example, in their response to *B. monnieri* where the percentage of escaped PINK1 flies increased significantly (52.1%,  $p < 0.01$ ) compared with that of control (15.3%), whereas in WT flies, the percentage of escaped flies decreased significantly (60.5%,  $p < 0.01$ ) compared with that of untreated flies (88.0%).

Levodopa significantly increased climbing ability in PINK1 flies (42.7%,  $p < 0.05$ ) compared with that in untreated flies (control, 15.3%). Zandopa (31.7%) treatment did not result in a significant effect on climbing ability in either untreated flies or flies treated with levodopa, despite containing approximately the same amount of levodopa as the levodopa treatment. In WT flies, on the other hand, levodopa (68.5%,  $p < 0.05$ ) treated flies had a significantly decreased climbing ability compared with Zandopa (93.2%) treated flies, although neither had a climbing ability significantly different from that of untreated flies (88.0%). Likewise, *B. monnieri* (52.1%) significantly increased climbing ability of PINK1 mutant flies compared with that of untreated (15.3%,  $p < 0.01$ ) flies.

Wild-type flies treated with *C. asiatica* (64.2% escaped) had a significantly lower percentage of escaped flies compared with that of the control (88.0%,  $p < 0.05$ ) and Zandopa (93.2%,  $p < 0.05$ ) treated flies. In PINK1 flies, *C. asiatica* (7.14%) treatment did not lead to a significant difference in escaped flies compared with that in untreated (15.3%) flies. However, the percentage was significantly lower in *C. asiatica* treated flies compared with that in flies treated with levodopa (42.7%,  $p < 0.01$ ), Zandopa (31.7%,  $p < 0.05$ ), *S. cordifolia* (35.9%,  $p < 0.01$ ), *B. monnieri* (52.1%,  $p < 0.01$ ) and the combination of all herbs (39.3%,  $p < 0.01$ ). On the other hand, vitamin C, Zandopa, *W. somnifera*, *S. cordifolia* and the combination of all herbs had no significant effects on climbing ability in either PINK1 or WT flies compared with that in the control.

## DISCUSSION

Our study shows that in the PINK1 flies, all treatments except *C. asiatica* increased climbing ability in the negative geotaxis test (albeit not always significantly so for some of the herbs as indicated in Fig. 1), whereas in WT flies, all treatments except Zandopa decreased climbing ability. This may be because in untreated WT flies, climbing ability is more or less optimal. In PINK1 flies, on the other hand, climbing ability is less than optimal owing to loss of function of PINK1, leading to muscle degeneration, hypersensitivity to

oxidative stress, mitochondrial defects, reduced lifespan and degeneration of dopaminergic neurons (Muñez-Soriano and Paricio, 2011). By treating PINK1 flies, one or more of these problems may be ameliorated, leading to a better ability to climb. For example, the presence of antioxidants from the herbs may decrease their sensitivity to oxidative stress, as PINK1 mutant flies are more sensitive to oxidative stress than healthy flies. Healthy WT flies are more likely to suffer from side effects of the treatments and not benefit from its pharmacological actions, as treatments may be more likely to push certain biochemical processes into a sub-optimal state, leading to a decreased climbing ability. For example, an important factor in the development of levodopa-induced dyskinesia seems to be the alternating highs and lows in levodopa plasma levels (Huot *et al.*, 2013). In PINK1 flies, these levodopa plasma levels have the benefit of improving locomotion, but in WT flies, they may only lead to dyskinesia, resulting in decreased climbing ability.

Ours results are in contrast with other studies comparing the effects of *M. pruriens* with equivalent amounts of levodopa (Kasture *et al.*, 2009) who found that in 6-OHDA-lesioned rats, acute administration of 16 mg/kg *M. pruriens* (containing 2 mg/kg levodopa) significantly improved motor function in several tests measuring akinesia and gait impairment, whereas a higher dose of levodopa (6 mg/kg) was needed to improve motor function to a similar extent. They also found that sub-chronic treatment with *M. pruriens* led to a lower dyskinetic potential than equivalent amounts of levodopa. This effect on dyskinesias may be partly due to 5-HT agonists present in *M. pruriens* (Kasture *et al.*, 2009). A similar study by Lieu *et al.* (2010) also using 6-OHDA-lesioned rats reported that *M. pruriens* induced significantly less dyskinesia compared with equivalent amounts of levodopa with peripheral DDC benserazide. The same study found that both *M. pruriens* extract and an equivalent amount of levodopa with benserazide significantly ameliorated parkinsonism in a stepping test and restored forelimb usage in the vibrissae-evoked forelimb placement test, whereas an equivalent amount of levodopa alone did not. Unfortunately, the researchers did not compare the effect of levodopa alone on dyskinesia. From these studies, it seems that treatment with levodopa leads to more dyskinesia than treatment with equivalent amounts of *M. pruriens*. These findings suggest that *M. pruriens* contains additional compounds that may improve locomotion, alter the properties of levodopa or alter levodopa's bioavailability (Kasture *et al.*, 2009). As highs and lows in levodopa plasma levels seem to play a major role in the development of dyskinesias (Huot *et al.*, 2013), another possibility is that treatment with *M. pruriens* leads to less dyskinesia compared with levodopa, because it may result in more constant plasma levels. Other compounds in *M. pruriens* may, for example, slow its uptake or breakdown.

Dyskinesia may have led to the (non-significantly) decreased climbing ability in WT flies, explaining the lower percentage of escaped WT flies when treated with levodopa. On the other hand, from these studies, it would be expected that PINK1 flies treated with Zandopa would also exhibit significant increases in percentage of flies that escaped compared with untreated flies or flies treated with levodopa. However, this was not observed. This may partly be explained by differences in study design, as these studies were conducted in a different type of animal and using a drug-induced parkinsonism model. Another explanation is that the dose of *M. pruriens* was too low to elicit a significant response. Less levodopa

than anticipated may have been present in HP-200 (Zandopa) owing to oxidation or degradation. Furthermore, it is possible that part of the positive effect of levodopa treatment was caused by the added vitamin C, which has been shown to delay the loss of climbing ability in a *D. melanogaster* PD model based on  $\alpha$ -synuclein (Khan *et al.*, 2012). Although vitamin C treatment (18.9% in vitamin C versus 15.3% in control) did not lead to a significant increase in climbing ability, it seemed to have a slight non-significant positive effect. *Mucuna pruriens* also contains vitamin C, but in the range of 35–45 mg ascorbic acid per 100 g (Kala and Mohan, 2010), which is many times smaller than the amount added to prevent oxidation in the levodopa treatment.

*Centella asiatica* had a negative effect on climbing ability in WT flies and no positive effect in PINK1 flies. Possible explanations are that *C. asiatica* is toxic to *D. melanogaster* or that the dose used was too high. However, no studies testing *C. asiatica* or its isolated compounds have been previously performed in *D. melanogaster*. Therefore, therapeutic dosage and toxicity of *C. asiatica* in *D. melanogaster* are unknown. Furthermore, only the effects of isolated compounds of *C. asiatica* on locomotion have been investigated in animal studies, only one of which tested its effect in a parkinsonism model (Xu *et al.*, 2013). Xu *et al.* (2013) reported that madecassoside isolated from *C. asiatica* significantly improved locomotion in a rat parkinsonism model. This is in contrast to findings of the present study. However, there are several important differences in study design, including the use of an isolated compound as opposed to whole aerial parts, rats instead of fruit flies, drug-induced versus genetic parkinsonism and differences in time of treatment in relation to disease induction. In the study described earlier, the test animals received treatment when they were fully developed, before and after parkinsonism was induced. Here, the flies received treatment while still developing. The exact mechanism whereby madecassoside works is unknown, but if it works by recovering the redox balance in tissues, like the authors proposed, then a sufficient level of the drug present in the body at the time of parkinsonism development may be important. All of these factors may have influenced the effect of *C. asiatica* on test animals suffering from parkinsonism. More research is needed to establish potential therapeutic effects of *C. asiatica* in *D. melanogaster*.

Two other studies (Nasir *et al.*, 2012; Wijeweera *et al.*, 2006) found that different compounds isolated from *C. asiatica*, asiatic acid and asiaticoside, respectively, had no significant effects on locomotor activity in healthy rats. Again, this is not in agreement with the present findings, where *C. asiatica* had a negative effect on climbing ability. For the same reasons mentioned earlier, these differences may be attributed to differences in study design. More research is needed to establish whether the observed negative effects in WT flies were due to toxicity of *C. asiatica* or if they were a genuine negative effect on climbing ability.

The results on *B. monnieri* are in agreement with those of a previous study by Hosamani and Muralidhara (2009) who reported that *B. monnieri* significantly improved climbing ability in rotenone-treated *D. melanogaster*. The same study found that *B. monnieri* inhibited dopamine depletion, decreased rotenone-induced mortality and protected against oxidative stress. Similar antiparkinsonian activities were reported in a *Caenorhabditis elegans* model of PD, where treatment with *B. monnieri* reduced  $\alpha$ -synuclein aggregation, prevented

dopaminergic neurodegeneration and restored lipid content (Jadiya *et al.*, 2011). Part of its effect may be explained by its protection against oxidative stress and damage, as demonstrated in several studies (e.g. Jyoti and Sharma, 2006; Hosamani and Muralidhara, 2010; Saini *et al.*, 2012; Shinomol and Muralidhara, 2011; Shinomol *et al.*, 2012).

*Bacopa monnieri* may act by enhancing the activities of a number of antioxidant enzymes, as was reported in healthy 3-month old female Wistar rats by Priyanka *et al.* (2013) and in 4-week old male mice reported by Shinomol and Muralidhara (2011). The latter also showed that *B. monnieri* extract improved redox status, as demonstrated by increased levels of reduced glutathione and thiol. Taken together, animal studies show that *B. monnieri* likely protects against oxidative damage, possibly by enhancing the activities of antioxidative enzymes and improving redox status. Several human studies have shown that it may also improve cognitive function (Roodenrys *et al.*, 2002, Barbhैया *et al.*, 2008, Stough *et al.*, 2008). In short, *B. monnieri* seems a promising new treatment for PD and other neurodegenerative disorders.

On the other hand, *B. monnieri* (60.5%) significantly decreased climbing ability in healthy WT flies compared with untreated flies (88.0%,  $p < 0.01$ ). No explanations for this could be found in the literature. The only previously performed study testing climbing ability in *B. monnieri*-treated fruit flies reported that *B. monnieri* by itself had no significant effect on locomotor activity (Hosamani and Muralidhara, 2009). It is possible that the dose was high enough for the healthy flies to induce negative effects. However, more research is needed to confirm this.

Vitamin C, Zandopa, *W. somnifera*, *S. cordifolia* and the combination of all herbs had no significant effects on climbing ability in either PINK1 or WT flies compared with that in the control. It is unknown whether this is because the dose was too low to elicit a response or because they had no properties able to alter locomotion, as for all of these treatments except vitamin C, no studies have been previously performed in *D. melanogaster*. As for vitamin C, in contrast to the current findings, Khan *et al.* (2012) reported that ascorbic acid at concentrations lower than the amount used in the present study was able to delay the loss of climbing ability in a *D. melanogaster* parkinsonism model based on  $\alpha$ -synuclein. The difference in outcomes may be due to the different model, or because a delay in loss of climbing ability is only observed after a longer period. At the time of testing, the flies were only approximately 1 or 2 days old.

## CONCLUSION

This study showed that *B. monnieri* significantly improved the climbing ability in a *D. melanogaster* PD model based on loss of function of PINK1. Combined with its potential to improve cognition and possibly prevent further cognitive decline as shown in human studies, this makes *B. monnieri* a promising alternative or complementary treatment. Further studies are required to establish optimal dosage and investigate possible causes of the observed negative effect on locomotion in the healthy WT flies. As the dosage used in the present study was a rough conversion of human dosage and only one dose was tested, it would be useful to establish dose–response curves for the effects of *B. monnieri* on climbing ability.



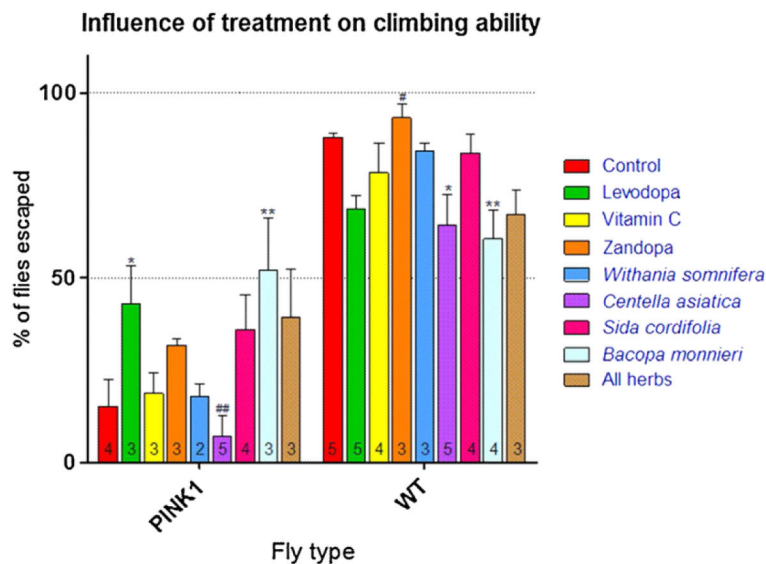
Additionally, a time course study of its effects on climbing ability or other measures of locomotion may provide valuable insights into its long-term effects and safety. It would also be interesting to measure the effects of *B. monnieri* on lifespan, as *B. monnieri* is seen as a rejuvenator in Ayurveda and may increase longevity (Williamson, 2002). The same investigations would also be interesting to perform in the other tested herbs, as from this study it is not possible to conclude whether they simply had no effect or if their dosage was not optimal. Human studies are also recommended to test whether *B. monnieri* has the same effect on locomotion in humans as in the *D. melanogaster* PD model used in this study.

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**Figure 1.**

Effects of different herbs or herbal preparations on the percentage of flies that escaped in the climbing ability test for flies with a loss of function PINK1 gene (PINK1) and wild-type (WT) flies. Vitamin C was added to levodopa to prevent oxidation. Flies of control groups underwent no treatment. The bars indicate the means and standard errors for each treatment. The numbers within bars indicate the number of replicates it is based on. Significant differences between the negative control (control) (denoted with stars (\*)) and the positive control (levodopa) (denoted with number signs (#)). \* $p < 0.05$ ; \*\* $p < 0.01$ . This figure is available in colour online at [wileyonlinelibrary.com/journal/ptr](http://wileyonlinelibrary.com/journal/ptr).

**Table 1**  
**Herbs and their dosage as used or recommended for human use, their equivalent per 100 g fly food as converted on the basis of Hong *et al.* (2011) and the purpose of the dose mentioned by the source**

Herb	Dosage in humans	Source	Equivalent (per 100 g fly food)	Purpose of use
<i>M. pruriens</i>	1.5–2.5 g	Williamson, 2002	2.1–3.5mg (2.4–4.0 mg Zandopa)	Several
	15–30 g (containing 10.7–21.3 g <i>M. pruriens</i> )	Katzenschlager, 2004	12.3–31.8 mg Zandopa	PD treatment
	22.5–67.5 g HP-200	HP-200 in Parkinson's Disease Study Group, 1995	31.5–94.5 mg Zandopa	PD treatment
<i>B. monnieri</i>	9 g	Nagashayana, 2000	12.6 mg (14.4 mg Zandopa)	PD treatment
	5–10 g powdered herb	Williamson, 2002	7–14 mg	Several
	300 mg KeenMind	Stough, 2008; Stough, 2012; Roodenrys, 2002	8.4 mg	Cognitive functioning, memory
<i>W. somnifera</i>	450 mg BacoMind	Barbhaiya, 2008; Morgan and Stevens, 2010	12.6 mg	Memory
	3–6 g	Williamson, 2002; WHO, 2009	4.2–8.4 mg	Several
	500 mg <sup>a</sup>	WHO, 2009	0.7 mg <sup>a</sup>	Stress
<i>C. asiatica</i>	600 mg <sup>a</sup>	Cooley, 2009	0.84 mg <sup>a</sup>	Anxiety
	29 g	Nagashayana, 2000	40.6 mg	PD treatment
	0.6 g	Williamson, 2002	0.84 mg	Several
<i>S. cordifolia</i>	1–2 g	WHO, 1999	1.4–2.8 mg	Several
	12 g	Bradwejn, 2000	16.8 mg	Anxiety associated with acoustic startle response
	0.5–2 g	Indigo Herbs, 2013	0.7–2.8 mg	Several
<i>S. cordifolia</i>	3–6 g	<a href="http://Singleherbs.org">Singleherbs.org</a> , 2013	4.2–8.4 mg	Several
	29 g	Nagashayana, 2000	40.6 mg	PD treatment

Taking into account that 150mg of KeenMind extract (Stough, 2008) or BacoMind extract (Abascal and Yarnell, 2011) is equivalent to 3 g dried herb.

<sup>a</sup>Unknown formulation could be concentrated extract or dried powdered herb and therefore left out of estimation of dosage needed for *D. melanogaster*.

**Table 2**  
**Dosages of herbs and Levodopa used, per 100 g fly food**

<b>Treatment</b>	<b>Dose (mg) per 100 g fly food</b>
<i>M. pruriens</i> (Zandopa)	25
<i>B. monnieri</i>	11
<i>W. somnifera</i>	23
<i>C. asiatica</i>	7
<i>S. cordifolia</i>	16
<i>Levodopa</i>	0.83

**Table 3**  
**ANOVA table showing the significance of the effects of treatment, fly type and interaction between treatment and fly type on percentage of flies that escaped**

Source of variation	Type III sum of squares	d.f.	Mean square	F-ratio	p value
Interaction	6701	8	838	4.38	0.0005
Treatment	4119	8	515	2.69	0.0157
Fly type	34 875	1	34 875	182	<0.0001
Residual	9188	48	191		

Number of missing values, 24.

d.f., degrees of freedom.