

Life Span and Motility Effects of Ethanolic Extracts from *Sophora moorcroftiana* Seeds on *Caenorhabditis elegans*

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Submitted: 21-05-2015

Revised: 26-12-2015

Published: 11-05-2016

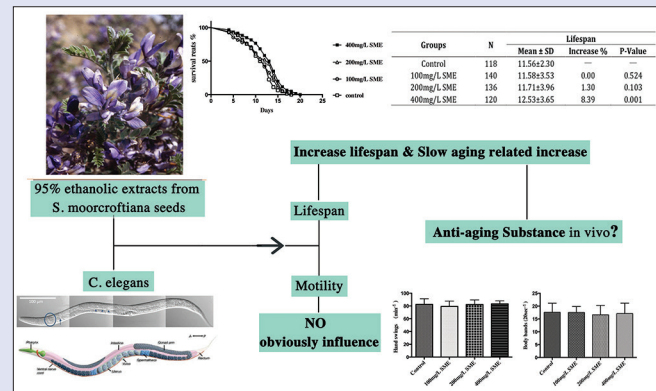
ABSTRACT

Background: *Sophora moorcroftiana* is an endemic shrub species with a great value in folk medicine in Tibet, China. In this study, relatively little is known about whether *S. moorcroftiana* is beneficial in animals' nervous system and life span or not. **Materials and Methods:** To address this question, under survival normal temperature (25°C), *S. moorcroftiana* seeds were extracted with 95% ethanol, and *Caenorhabditis elegans* were exposed to three different extract concentrations (100 mg/L, 200 mg/L, and 400 mg/mL) from *S. moorcroftiana* seeds. **Results:** The 95% ethanolic extracts from *S. moorcroftiana* seeds could increase life span and slow aging-related increase in *C. elegans* and could not obviously influence the motility of *C. elegans*. **Conclusion:** Given these results by our experiment for life span and motility with 95% ethanolic extracts from *S. moorcroftiana* seeds in *C. elegans*, the question whether *S. moorcroftiana* acts as an anti-aging substance *in vivo* arises.

Key words: Anti-aging, *Caenorhabditis elegans*, motility, *Sophora moorcroftiana*

SUMMARY

- The 95% ethanolic extracts from *S. moorcroftiana* seeds have no effect on the life span in *C. elegans* when extract concentrations from *S. moorcroftiana* seeds <400 mg/L
- The 400 mg/L 95% ethanolic extracts from *S. moorcroftiana* seeds could increase life span in *C. elegans*
- The 95% ethanolic extracts from *S. moorcroftiana* seeds could not obviously influence the motility in *C. elegans*.



Abbreviation used: *S. moorcroftiana*: *Sophora moorcroftiana*; *C. elegans*: *Caenorhabditis elegans*; *E. coli* OP50: *Escherichia coli* OP50; DMSO: Dimethyl sulfoxide.

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DOI: 10.4103/0973-1296.182152

Access this article online

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INTRODUCTION

Sophora moorcroftiana is an endemic shrub species in Tibet, China, and is mainly distributed in the wide valleys in the middle reaches of several main tributaries of the Yalu Tsangpo River. *Sophora moorcroftiana* seeds have been used for a long time in Chinese folk medicine. The decoction of the seed has been used in Chinese folk medicine for dephlogistication (translated, in general, as anti-inflammation in modern medicine), detoxication, emetic processes, and treating infectious diseases and verminosis.^[1] The alkaloids obtained from *S. moorcroftiana* seeds have a protoscolicidal, anti-inflammatory effect^[2] and induce apoptosis of the human stomach cancer SGC-7901 cell line *in vitro*.^[3] As a magical medicinal plant, which has not been fully studied, *S. moorcroftiana* may have more unknown available value *in vivo*. One of these organisms which can be used for life span and nervous system studies is *Caenorhabditis elegans*, which is readily available, easy to culture in the laboratory, has a short life span, and vast knowledge is known about this nematode.^[4-6] Moreover, other studies have shown that some compounds can prolong *C. elegans* life span

and influence motility under laboratory conditions.^[7] In light of these studies, we aimed to determine the effects of different concentrations of 95% ethanolic extracts from *S. moorcroftiana* on life span and motility in *C. elegans*.

In this study, it can be reported that 95% ethanolic extracts from *S. moorcroftiana* seeds could increase life span and slow aging-related increase in *C. elegans*. In addition, 95% ethanolic extracts from *S. moorcroftiana* seeds have no effect on the motility in *C. elegans*.

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Cite this article as: Li X, Han J, Zhu R, Cui R, Ma X, Dong K. Life span and motility effects of ethanolic extracts from *Sophora moorcroftiana* seeds on *Caenorhabditis elegans*. Phcog Mag 2016;12:S228-30.

Together, our study suggests the existence of an anti-aging compound within ethanolic extracts from *S. moorcroftiana* seeds.

MATERIALS AND METHODS

Caenorhabditis elegans and its synchronization

The *C. elegans* wild-type (N2) strain and its food source *Escherichia coli* OP50 strain were kindly provided by Professor Li Hongyu, who obtained these from *Caenorhabditis* Genetic Center at the University of Minnesota (USA). A volume of 200 μ L of OP50 (overnight culture) was dropped at the center of 60 mm nematode growth medium (NGM) plates, which were allowed to dry overnight before worms were transferred. *C. elegans* cultivation media supplies were purchased from Solarbio (China).^[8]

L1 larvae were prepared by eggs synchronization, hermaphrodites were lysed in 20% bleach, 0.5 M NaOH, until fragmented, and eggs were incubated in M9 buffer without food overnight (14–18 h) at 20°C with agitation (150 rpm), to allow larvae to hatch and arrest the development because of starvation. About 600 worms (in 1.5 μ L total volume of M9 buffer) were placed in several drops on each assay plate (60 mm across) with enriched NGM, seeded with *E. coli* OP50.^[9]

Sophora moorcroftiana extracts preparation

The 95% ethanolic extracts were extracted from *S. moorcroftiana* seeds by our laboratory as described previously.^[10] The 95% ethanolic extracts were tested for the presence of carbohydrates, proteins, amino acids, alkaloids, flavonoids, glycosides, saponins, quinones, and fats and oils, using standard procedures.^[11,12] The dry crude extracts of *S. moorcroftiana* were dissolved into the required concentrations in dimethyl sulfoxide (DMSO); the final concentration of DMSO was <2%.^[13] The extracts were diluted with distillate water when it was used for experiment.

Life span assay

The life span assays were performed according to the standard protocol recently described by Swain *et al.*^[14] The animals were kept at 25°C during growth into adult stage and then treated for 2 h with the agents tested, which was composed of distillate water and extracts (dissolved in DMSO) in proportion to the required concentration. Afterward, they were fed in NGM agar plates spotted with OP50 for life span assays. Life span assays were performed at 25°C by checking the animals at each day at a fixed time until all the animals died. The escaping animals from the Petri dishes were excluded from the study. The study was planned as four groups. One group was used as control. The others were 100 mg/L, 200 mg/L, and 400 mg/L concentrations of 95% ethanolic extracts from *S. moorcroftiana*, respectively. Then, worms were incubated at 25°C. Animals were counted and recorded on the day. Each experiment was done in replicates of three. Data are presented as mean \pm standard deviation (SD). All statistical analyses were carried out using SPSS software (IBM SPSS Statistics, Armonk, NY, USA). Nonparametric tests were carried out, and *P* values were calculated using the Wilcoxon test; *P* < 0.05 was accepted as statistically significant.

Motility assay

The motility assays were performed according to the standard protocol recently described by Tsalik and Hobert.^[15] The feeding ways and treated mode by agents tested of *C. elegans* are the same as the life span assay in this experiment. However, they were fed in NGM agar plates without OP50 for assays after treatment in agents tested. The body bends per 20 s and the head swings per minute were counted with zoom stereo microscope. Each experiment was done in replicates of three. Data are presented as mean \pm SD. The data were pooled and analyzed using Student's *t*-test; *P* < 0.05 was accepted as statistically significant.

RESULTS AND DISCUSSION

The 95% ethanolic extracts from *Sophora moorcroftiana* seeds can increase life span and slow aging-related increase on *Caenorhabditis elegans*

Although the alkaloids obtained from *S. moorcroftiana* have a protoscolicidal, anti-inflammatory effect and induce apoptosis of the human stomach cancer SGC-7901 cell line *in vitro*,^[2,3] no previous literature has shown the effectiveness of crude extracts of *S. moorcroftiana* seeds on anti-aging and the nervous system. Our data demonstrated that the high concentration (400 mg/L) of 95% ethanol extract from *S. moorcroftiana* seeds were significantly active in the dilation of life span [Table 1] and could effectively slow down aging-related increase in *C. elegans* [Figure 1]. There is no concrete evidence for whether the effectiveness of 95% ethanolic extracts from *S. moorcroftiana* on life span depends on the concentration.

Sophora moorcroftiana extracts have no obvious influence in motility of *Caenorhabditis elegans*

Motility is one of the key physiological indexes to assess the excitability of animals' nervous system. The body bends per 20 s and the head swings per minute are specific testing items of motility of *C. elegans*, and generally speaking their results are similar. Our data (both the body bends and the head swings) demonstrated that the effects of all experiment groups were not significantly different from those of control group [Figure 2]. In other words, 95% ethanol extracts from *S. moorcroftiana* seeds were not obviously effective in motility of *C. elegans*.

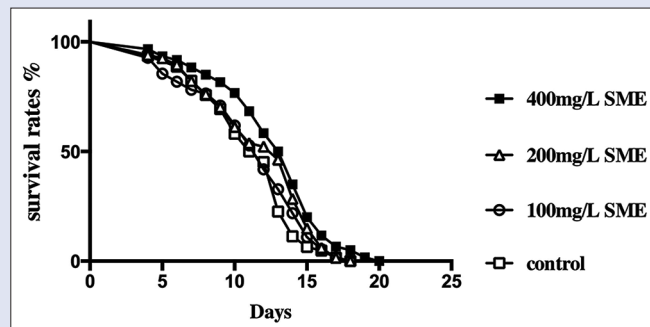


Figure 1: The survival curve of *Caenorhabditis elegans* at 25°C with various interventions. The supplementation with the high concentration (400 mg/L) of 95% ethanol extract from *Sophora moorcroftiana* seeds results in more prolonged life span extension in *Caenorhabditis elegans*. Each life span experiment was repeated at least three independent times with similar results

Table 1: The life span of *Caenorhabditis elegans* at 25°C with various interventions

Groups	n	Life span		
		Mean \pm SD	Increase percentage	P
Control	118	11.56 \pm 2.30	-	-
100 mg/L SME	140	11.58 \pm 3.53	0.00	0.524
200 mg/L SME	136	11.71 \pm 3.96	1.30	0.103
400 mg/L SME	120	12.53 \pm 3.65	8.39	0.001

The life span data were analyzed using the Wilcoxon test and *P* values for each experiment are shown. Life span and SD are shown in days. SME: *Sophora moorcroftiana* extracts; n: The number of total effective *Caenorhabditis elegans* in each group; SD: Standard deviation

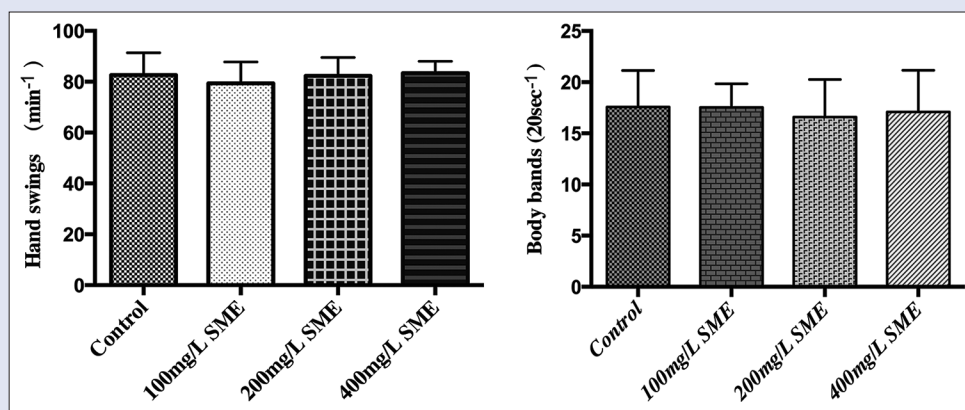


Figure 2: Motility of *Caenorhabditis elegans* with various interventions. The body bends per 20 s and the head swings per minute are the main outcome measures of the motility of *Caenorhabditis elegans*. We randomly selected 20 worms to observe in each experiment group. Each of the motility assays was repeated at least three independent times with similar results. *P* value was calculated using Student's *t*-test

CONCLUSION

We explored the potential application of 95% ethanolic extracts from *S. moorcroftiana* seeds in the present study. On the basis of our experimental evidence, we are the first to suggest that 95% ethanol extracts from *S. moorcroftiana* seeds could increase life span and could not obviously influence the motility in *C. elegans*. Therefore, it could be a new source of natural, which will play an important role in anti-aging. However, in terms of which ingredients of the crude 95% ethanol extracts of *S. moorcroftiana* seeds have effect on anti-aging and why this substance could influence life span, further studies are needed to explore related important issues. Noticeably, however, if the similar results would occur in humans still remain unknown.

Acknowledgments

The authors are grateful to Dr. Dejuan Zhi of Microbial and Biochemical Pharmacy laboratory in Lanzhou University for helpful discussions.

Financial support and sponsorship

The National Natural Science Foundation of China (31360604) and Lanzhou University Innovation and Entrepreneurship Fund for College Students (20141073001313).

Conflicts of interest

There are no conflicts of interest.

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