**Withania somnifera** root extract extends lifespan of *Caenorhabditis elegans*

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**Key Words**

*C. elegans*  
Lifespan extension  
Ashwagandha  
Withanolides

**Abstract**

Background: In the ancient Indian herbal medicine system several ayurvedic preparations are claimed to have longevity enhancing effects. But, so far, no clear scientific evidence has been provided. One among them, is the roots of the plant, commonly known as Ashwagandha (*Withania somnifera* Dunal- WSD), which is supposed to have myriad of beneficial effects including long life. Purpose: Here, we evaluated both the root extract (RE) and its purified ingredients (PI-RE) with a similar composition as in RE obtained from the roots of WSD for lifespan extension in the well established model system, *C. elegans*. PI-RE could extend the lifespan of *C. elegans*. Methods: We used wild type *C. elegans* (N2) or RB918: acr-16 (ok789); and NL2099: rrf-3 (pk1426) mutant worms and analysed their lifespan assay in Ashwagandha extract spreaded on plates containing Bacterial Lawns. Results: Strangely, while there was no effect on the wild type worms, the mutant for the human nicotinic acetylcholine receptor, nAChR, α7 equivalent, acr-16, showed around ~20% lifespan extension when treated with PI-RE. Conclusion: Thus, we are able to show that one of the age old healthy longevity supplements, Ashwagandha does extend lifespan of *C. elegans*.

**Introduction**

Aging is a complex, pleiotrophic phenomenon yet to be fully understood. Ever being youthful (anti-aging) and prolonged healthy living without diseases is a quest from time immemorial. Identification of long-lived mutants in *C. elegans* opened the avenue for healthy lifespan extension research.⁴⁻³ The major challenge with lifespan extension studies is the long time requirement (in months and years) in the mammalian systems. This could be circumvented using the versatile, seemingly simple model organism, *C. elegans*, which has a short lifespan (in days), but, fundamental mechanisms and systems amazingly similar to the mammalian system.⁴ Further, the increase in longevity mechanisms identified in *C. elegans* is shown to follow similar pattern in flies,⁵ mice⁶ and humans.⁷⁻⁸ The positive correlation between reduced insulin/IGF-1 signaling's contribution to lifespan and protection against Alzheimer's disease has been shown to provide antioxidant⁹ and anti-inflammatory effects¹⁰ and protection against Alzheimer's disease.¹⁰ Ashwagandha has minimal side effects and is in use for thousands of years.¹⁶,¹⁸ This is also implicated to have longevity enhancing effects but not clearly demonstrated.

Here in this report, we evaluated the RE extract prepared from the roots of *Withania somnifera* (ixoreal Biomed Pvt Ltd, India) and the purified components in it for the effect on lifespan extension in *C. elegans*. We find that indeed the purified fraction can extend *C. elegans* lifespan.

**Methods**

*Withania somnifera* strains

The *C. elegans* wild type (N2) or RB918: acr-16 (ok789); and NL2099: rrf-3 (pk1426) mutant worms were grown on NGM plates with OP50 *E. coli* as food at 20°C following standard protocols.²²

Preparation of Ashwagandha root extract (RE) containing plates: We followed the protocol of spreading of the RE and purified ingredients on the OP50 bacterial lawn containing NGM plates. We diluted Ashwagandha extract-RE (IXOREAL, Hyderabad, INDIA) of various fractions: i) suspension ii) ~80% water soluble fraction iii) ~60% water soluble fraction and iv) ethanol solubilised mixture of purified components in the same ratio as in RE. The required concentration dilution was done in M9 buffer and spread on the plates containing bacterial lawn with two controls i) no vehicle and ii) with ethanol as the vehicle.

Lifespan assays: Approximately 30–40 embryos were transferred to 35 mm plate with *E. coli* OP50 bacterial lawn in NGM agar with or without the compound and maintained at 25°C. The lifespan assay was carried out as described earlier with minor modifications. Once the worms reach the adult stage, we shift them every day to fresh plates till the end of reproduction. After that we transfer them to fresh plates every two or three days. The lifespan was counted starting L4 stage which was considered as 0. The worms which crawled off the plates were excluded. The worms were considered dead when they did not move at all to prodding with the platinum wire. We carried out the initial screening once...
or twice and the positive fraction assay was repeated at least two to three times. Survival Graph and statistical analyses were done using Sigmaplot 10.0. and SigmaStat 3.5.

Results and Discussion

Purified ingredients (PI) mixture of Root extract (RE) extends lifespan in C. elegans

We screened C. elegans for lifespan extension following the published protocol.11 Briefly, we treated the worms chronically with various RE and its fractions from embryo stage till death. We have screened crude (Fig. 1) and water soluble fractions of Ashwagandha (sample 1–80% water soluble and sample 2–60% water soluble) (Fig. 2) and the purified mixture with the composition (Fig. 2) as in PI-RE (Fig. 3). We carried out a dose response analysis (Fig. 3A) and found that PI-RE at a concentration of 100 ng/ml is able to extend the lifespan in the human nicotinic acetylcholine receptor α7 equivalent mutant, acr-16, but not in the wild type or another mutant which shows enhanced RNAi, rrf-3, suggesting RE can extend C. elegans lifespan in a specific

![Fig. 1: Evaluation of RE for lifespan extension of acr-16 mutant of C. elegans. A. Crude extract: Black line- NGM- (N = 112); Black dash- RE – 100 µg/ml (N = 131); Grey dash - and 1 mg/ml (N = 77). B. Water soluble: Black line- NGM- (N = 37); Black dash- RE (80% water soluble) – 100 µg/ml (N = 35); Grey dash RE (80% water soluble) – 100 µg/ml (N = 34).

![Fig. 2: Active compounds of RE – HPLC trace.]
background. The PI-RE treated and the control worm’s median
days are shown in Table 1. PI-RE showed 21.4% lifespan 
extension compared to the control (NGM medium) and 
~14% in comparison to the vehicle (ethanol).

It is intriguing that PI-RE did not show lifespan extension 
in the wildtype worms or the enhanced RNAi mutant, rrf-3, 
but extended the lifespan of the nAchR, α7, subunit mutant, 
acr-16. Despite the reports that ashwagandha is given for 
healthy longevity in ayurvedic medicine, there is no concrete 
evidence for the same. Here we show that Ashwagandha can 
indeed extend the lifespan of C. elegans adding credence 
to this report.

Various supplements and drugs are being evaluated for in-
duction of lifespan extension in worms. Once identified in the 
worms, they can/are further screened in the mammalian model-
mice. The search to find an ideal drug/compound, which could 
extend lifespan, is ongoing. Polyphenols in blueberry and res- 
veratrol originally identified in the skin of the black grapes 
and reserpine from the plant Raulwolfia serpentine can ex-
tend C. elegans lifespan. Now, we find that another ayurvedi-
cally used herbal plant, Aswagandha can extend lifespan (Fig. 3B). Interestingly, the vehicle ethanol per se is able to cause 
marginal lifespan extension. In terms of drugs, the antiepileptic 
drug, trimethadione, the antidepressant mianserin and the 
antihypertensive drug, reserpine, extends the worm lifespan.

In addition, Rapamycin is shown to extend lifespan in worms 
and mice. However the side effects need to be taken into con-
sideration. Hence, it will be worthwhile to evaluate KSM-66 for 
longevity effects on higher animal model.

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Table 1: PI-RE treatment extends C.elegans (acr-16 mutant) 
lifespan

<table>
<thead>
<tr>
<th>Sample</th>
<th>Median Lifespan + Std. Dev</th>
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<tbody>
<tr>
<td>NGM</td>
<td>14 ± 3 (109)</td>
</tr>
<tr>
<td>NGM + ethanol</td>
<td>15 ± 1.3 (127)</td>
</tr>
<tr>
<td>NGM + PI-KSM- 100 ng/ml</td>
<td>17 ± 3.14 (213)</td>
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</tbody>
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Fig. 3: Evaluation of purified compounds mixture-PI-RE for lifespan extension of acr-16 mutant of C. elegans. A. Dose response : Black line- NGM- (N = 39); Black dash- PI-RE – 5 ng/ml (N = 40); Grey long dash - 10 ng/ml (N = 40); Grey short dash – 100 ng/ml (N = 50). B. PI-RE: Black line- NGM- (N = 109); Grey dash- Ethanol (N = 127) PI-RE 100 ng/ml (N = 213).


