Supplementary File

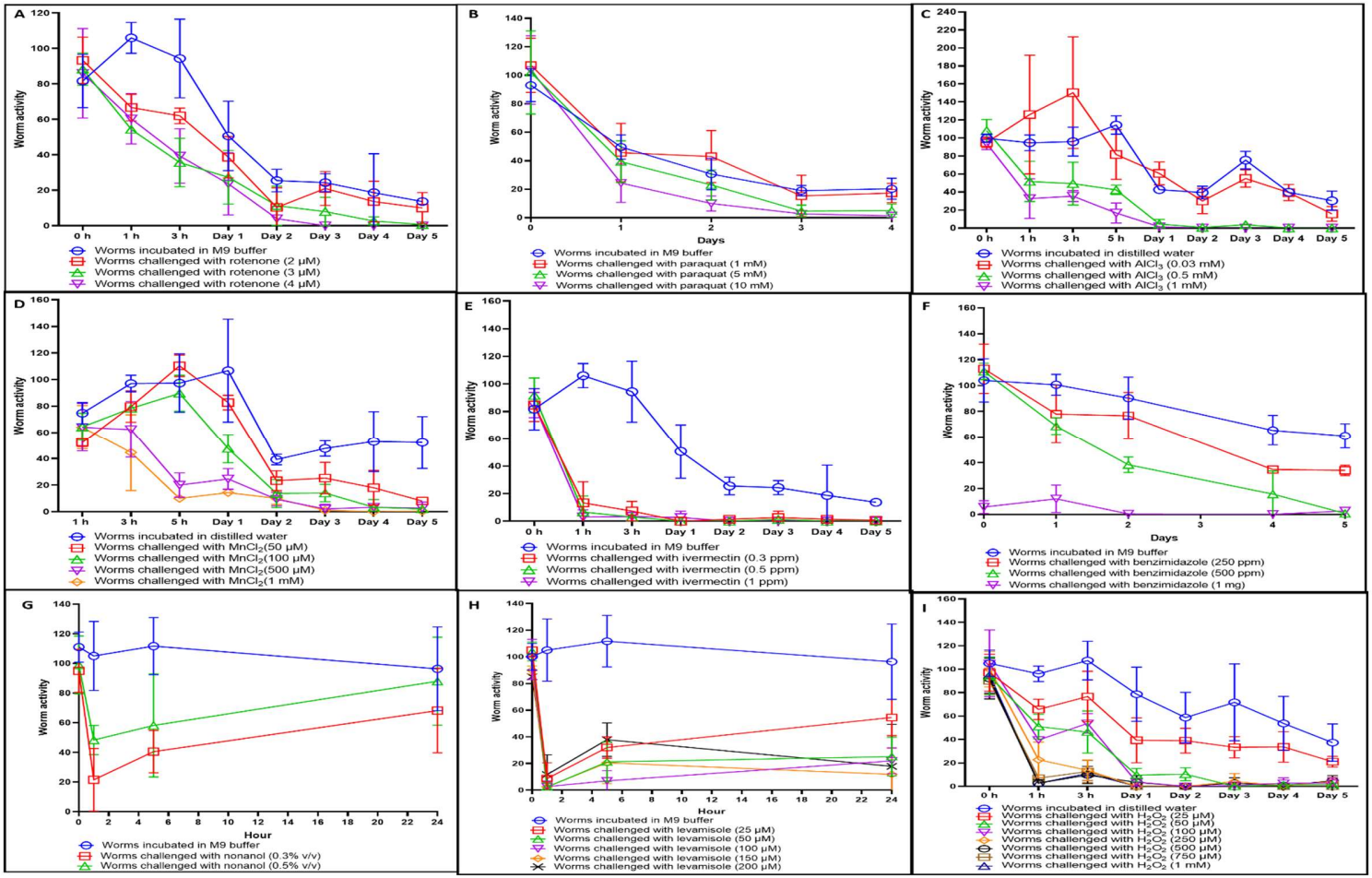

Figure S1. Effect of toxic chemicals on *C. elegans* (A) Rotenone **(B)** Paraquat **(C)** Aluminum Chloride ($AlCl_3$) **(D)** Manganese chloride ($MnCl_2$) **(E)** Ivermectin **(F)** Benzimidazole **(G)** Nonanol **(H)** Levamisole **(I)** Hydrogen peroxide (H_2O_2). Population size of worms per well was approximately 100.

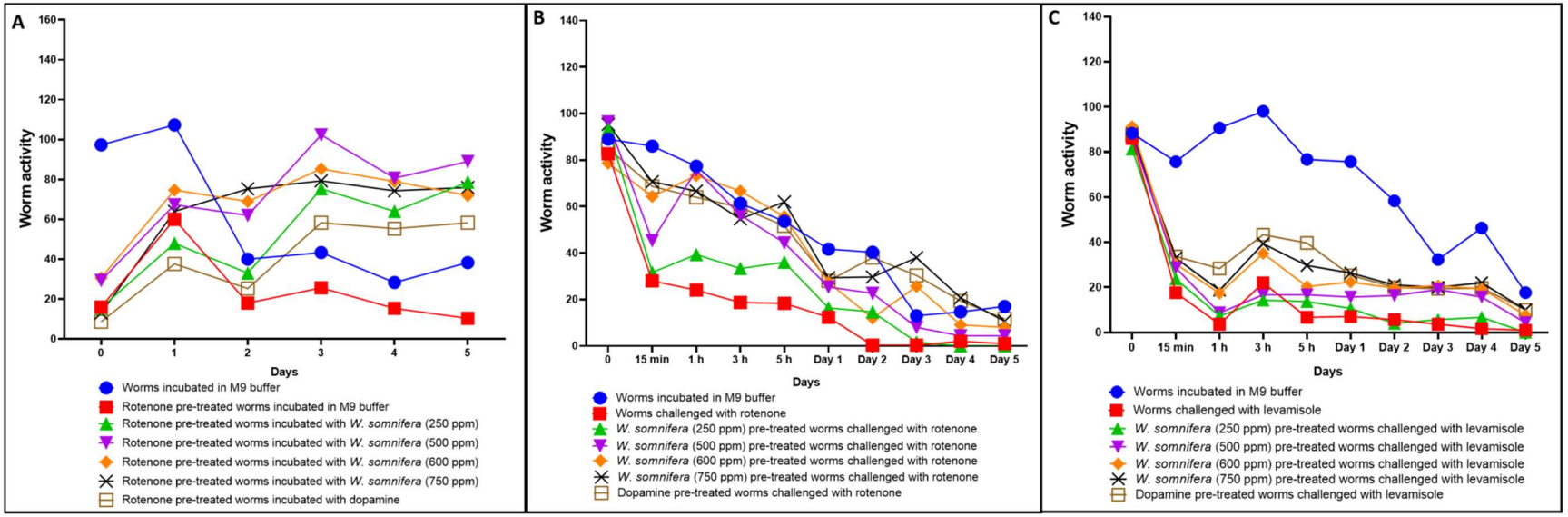

Figure S2. Effect of *Withania somnifera* root extract (WSRE) at different concentrations on worm activity in face of challenge with chemical stressors. (A) Dose-response evaluation with respect to recovery from rotenone-mediated shock. All WSRE concentrations were statistically at par. Few progenies were observed in wells pertaining to 600 and 750 ppm of WSRE. **(B)** Dose-response study with respect to the prophylactic effect of WSRE pre-exposure. When worms pre-exposed to different concentrations of WSRE were subsequently challenged with continuous presence of rotenone, for first 24 h, WSRE concentrations ranging from 500-750 were statistically at par with respect to their toxicity-alleviating effect. Day-three onward, only 600 ppm and 750 ppm could exert protective effect. The latter higher concentration was not more effective ($p=0.20$) than the former. **(C)** When worms pre-exposed to different concentrations of WSRE were subsequently challenged with continuous presence of levamisole, on fourth and fifth day, worm motility in wells corresponding to WSRE concentrations 600-750 ppm was better ($p\leq0.005$) than control, but at par ($p\geq0.33$) between themselves.

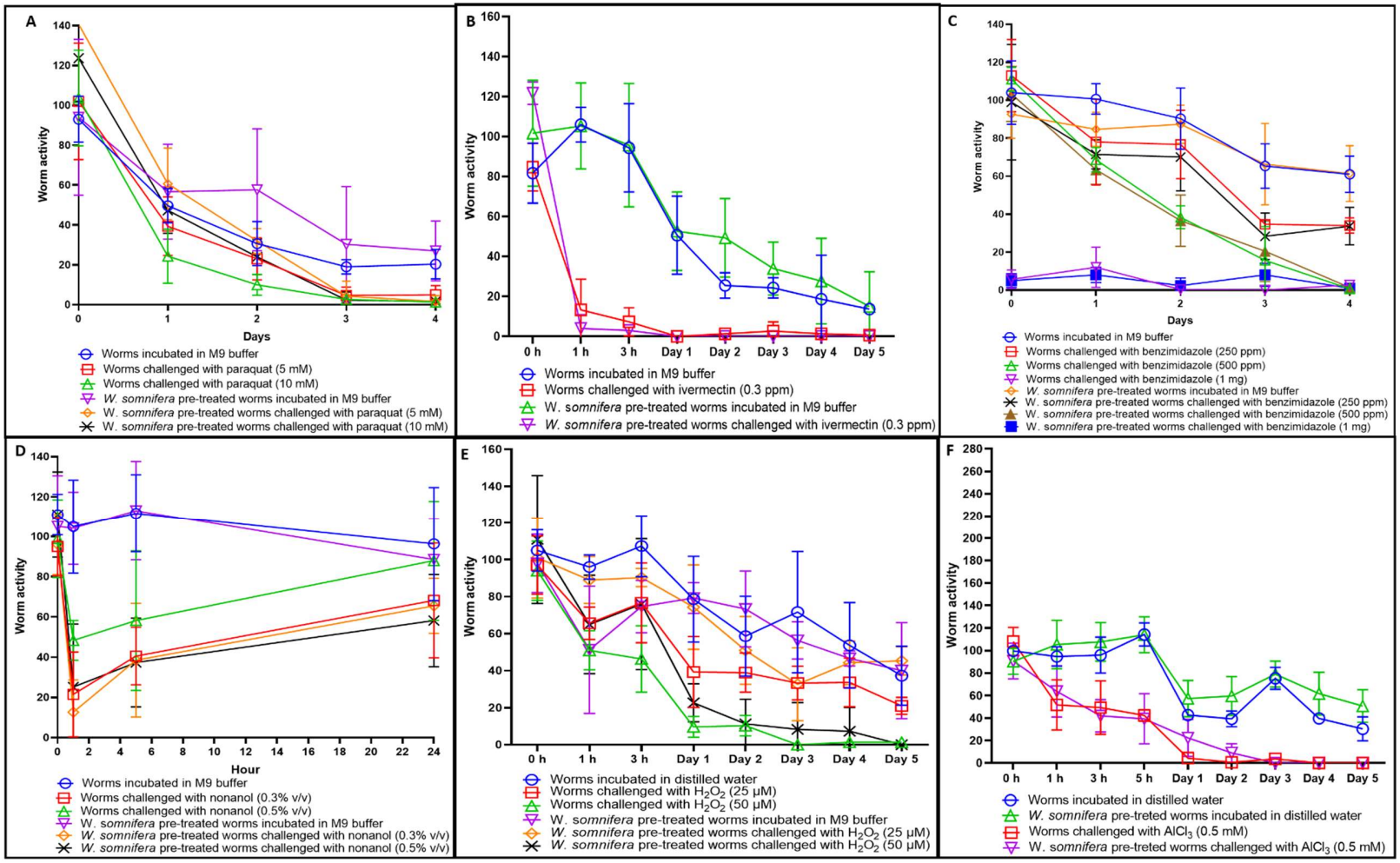

Figure S3. Pre-treatment with *Withania somnifera* did not protect worms from subsequent challenge with (A) Paraquat **(B)** Ivermectin **(C)** Benzimidazole **(D)** Nonanol **(E)** Hydrogen peroxide (H_2O_2) **or (F)** Aluminum Chloride ($AlCl_3$)

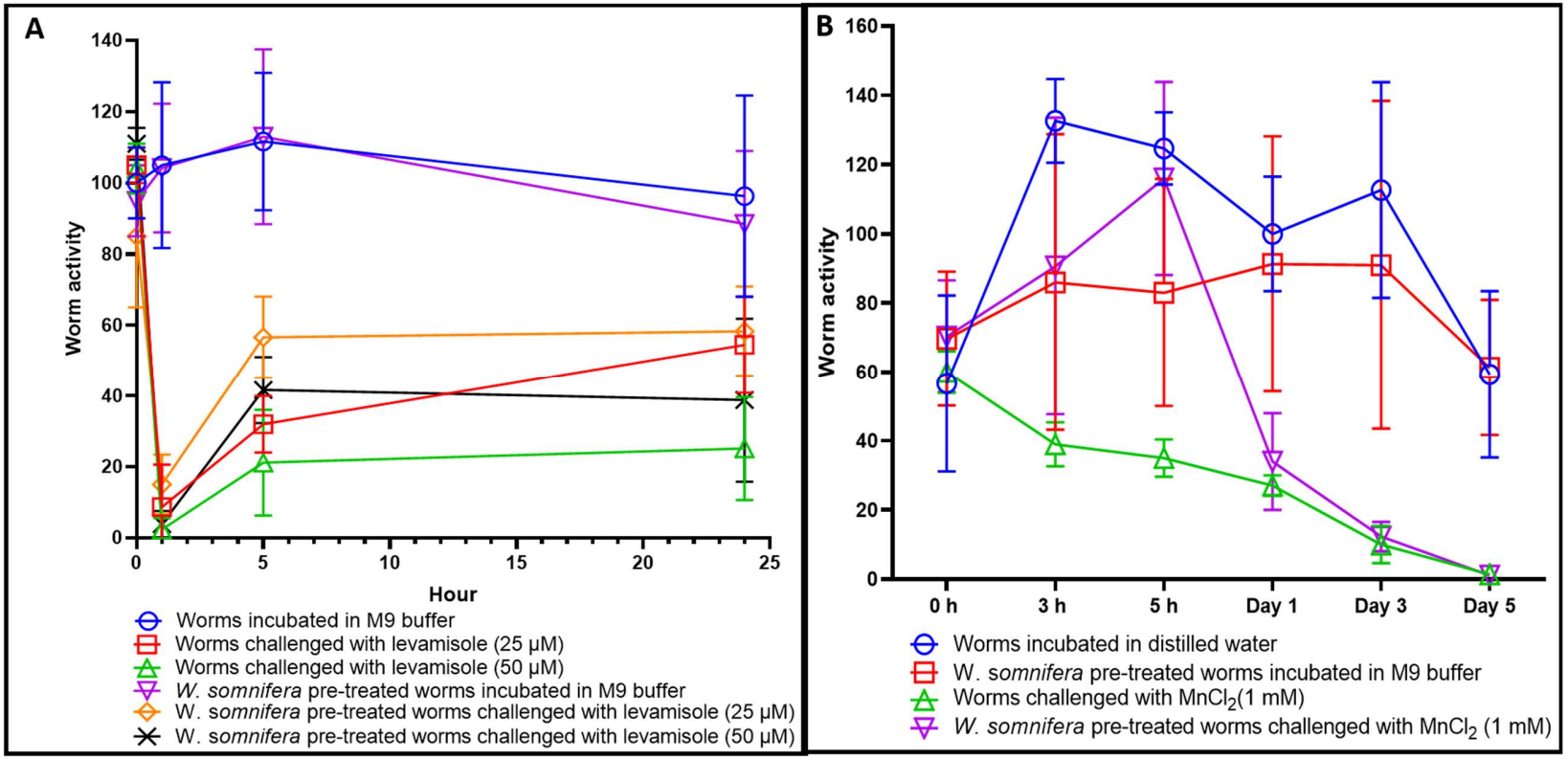

Figure S4. *Withania somnifera* offers prophylactic protection to worms subsequently challenged with stressors. (A) Levamisole. Worms were pre-fed for 48 h with WSRE, followed by continuous exposure to levamisole. Pre-feeding with WSRE supported higher worm activity compared to the levamisole control till 5 hour. After that worm activity was statistically equal in all wells (data not shown). **(B) Manganese chloride ($MnCl_2$).** Worms were pre-fed for 48 h with WSRE, followed by continuous exposure to $MnCl_2$. Pre-feeding with WSRE delayed the death in worm population.

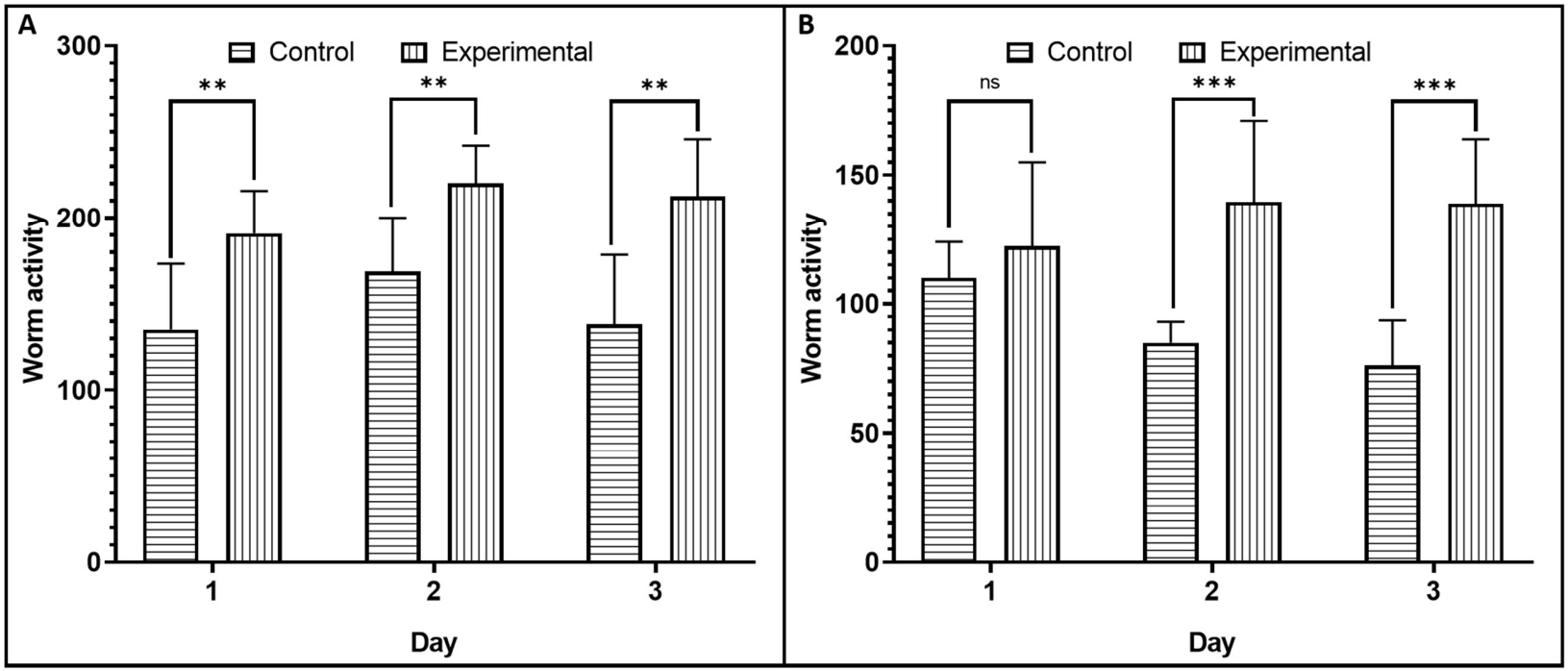

Figure S5. *Withania somnifera* extract retains its beneficial effect on worm healthspan even after one year of refrigerated storage. (A) Study conducted in July 2024. **(B)** Study conducted in August 2025**.** WSRE dissolved in water, filter-sterilized, and stored under refrigeration in glass vial**.** Population size of worms was 100 per well. $^{**}p \leq 0.01$, $^{***}p \leq 0.001$

Table S1-S7 contains the mean values of worm activity counts as measured by the worm tracker, from which the figures provided in the main manuscript were generated.

Table S1. Protective effect of *Withania somnifera* against rotenone [Raw data pertaining to Figure 1(A)]

Group	Treatment	0 h	15 min	1 h	3 h	5 h	Day 1	Day 2	Day 3	Day 4	Day 5
A	Worms incubated in M9 buffer	88.67 ± 8.14	128.33 ± 16.46^{b}	124.00 ± 29.64^{b}	116.17 ± 20.06^{bc}	130.50 ± 25.62^{bc}	69.00 ± 22.14^{b}	48.17 ± 13.96^{bc}	36.83 ± 9.81^{bcd}	28.83 ± 13.59^{b}	19.83 ± 4.07^{bcd}
B	Worms challenged with rotenone	88.83 ± 11.65	47.00 ± 16.25^{acd}	44.17 ± 10.15^{acd}	42.67 ± 12.23^{acd}	38.50 ± 10.09^{acd}	15.17 ± 5.19^{acd}	5.17 ± 5.74^{acd}	3.50 ± 4.18^{acd}	2.67 ± 4.13^{acd}	0.00 ± 0.00^{acd}
C	*W. somnifera* pre-treated worms challenged with rotenone	99.33 ± 13.60	96.00 ± 21.14^{b}	101.33 ± 13.75^{b}	70.83 ± 8.28^{abd}	61.17 ± 11.36^{ab}	39.17 ± 5.85^{bd}	24.67 ± 11.50^{ab}	12.50 ± 1.76^{abd}	10.67 ± 3.93^{bd}	5.33 ± 1.86^{abd}
D	Dopamine pre-treated worms challenged with rotenone	90.33 ± 20.88	120.67 ± 14.14^{b}	116.00 ± 30.71^{b}	108.50 ± 14.52^{bc}	92.50 ± 31.87^{b}	57.00 ± 6.07^{bc}	37.67 ± 9.29^{b}	21.83 ± 3.54^{abc}	20.83 ± 5.56^{bc}	12.67 ± 3.20^{abc}

Superscript letters denote a statistically significant difference ($p < 0.05$) from the indicated group within the same column. [a] indicates significantly different from Group A; [b] indicates significantly different from Group B; [c] indicates significantly different from Group C; [d] indicates significantly different from Group D.

Table S2. Protective effect of *Withania somnifera* against rotenone [Raw data pertaining to Figure 1(B)]

Group	Treatment	0 h	Day 1	Day 2	Day 3	Day 4	Day 5
A	Worms incubated in M9 buffer	111.11 ± 39.51^{bcde}	110.00 ± 14.10^{b}	85.00 ± 8.14^{bd}	76.22 ± 17.51^{bce}	67.33 ± 13.09^{be}	62.78 ± 15.54^{bce}
B	Rotenone pre-treated worms incubated in M9 buffer	37.56 ± 20.11^{a}	64.67 ± 18.29^{ace}	49.78 ± 11.63^{ace}	46.00 ± 15.09^{ace}	31.44 ± 12.12^{ace}	24.33 ± 12.47^{acde}
C	Rotenone pre-treated worms incubated with *W. somnifera*	55.89 ± 34.45^{a}	132.44 ± 51.64^{b}	145.33 ± 54.64^{bde}	133.89 ± 45.13^{abd}	119.78 ± 47.25^{bd}	135.11 ± 46.47^{abd}
D	Rotenone pre-treated worms incubated with cortisol	34.22 ± 13.27^{a}	90.00 ± 30.96	65.78 ± 12.87^{ac}	62.11 ± 11.13^{ce}	52.33 ± 17.16^{ce}	47.00 ± 11.16^{bce}
E	Rotenone pre-treated worms incubated with dopamine	36.00 ± 16.82^{a}	105.00 ± 20.78^{b}	75.44 ± 9.06^{bc}	108.22 ± 16.20^{abd}	126.22 ± 30.31^{abd}	201.89 ± 78.50^{abd}

Superscript letters denote a statistically significant difference ($p < 0.05$) from the indicated group within the same column. [a] indicates significantly different from Group A; [b] indicates significantly different from Group B; [c] indicates significantly different from Group C; [d] indicates significantly different from Group D; [e] indicates significantly different from Group E. Shaded cells ( ) indicate presence of progenies, who also contributed towards the measured activity count.

Table S3. Protective effect of *Withania somnifera* against levamisole [Raw data pertaining to Figure 3(A)]

Group	Treatment	0 h	15 min	1 h	3 h	5 h	Day 1	Day 2	Day 3	Day 4	Day 5
A	Worms incubated in M9 buffer	88.67 ± 8.14	128.33 ± 16.46^{bcd}	124.00 ± 29.64^{bcd}	116.17 ± 20.06^{bcd}	130.50 ± 25.62^{bcd}	69.00 ± 22.14^{bc}	48.17 ± 13.96^{bc}	36.83 ± 9.81^{bc}	28.83 ± 13.59^{bc}	19.83 ± 4.07^{bcd}
B	Worms challenged with levamisole	95.00 ± 12.98	13.33 ± 8.76^{ad}	2.50 ± 3.89^{ad}	12.17 ± 6.91^{acd}	16.33 ± 8.02^{acd}	9.67 ± 2.58^{ad}	5.83 ± 3.71^{ad}	3.67 ± 2.16^{acd}	2.67 ± 2.16^{ad}	3.00 ± 2.83^{ad}
C	*W. somnifera* pre-treated worms challenged with levamisole	120.67 ± 44.88	23.17 ± 5.31^{ad}	15.00 ± 10.20^{a}	30.83 ± 8.06^{ab}	39.83 ± 7.08^{ab}	17.33 ± 5.92^{a}	10.67 ± 4.08^{ad}	13.83 ± 4.17^{abd}	7.00 ± 3.03^{ad}	7.17 ± 1.72^{a}
D	Dopamine pre-treated worms challenged with levamisole	111.17 ± 36.88	49.83 ± 14.97^{abc}	24.00 ± 12.96^{ab}	34.17 ± 8.13^{ab}	44.50 ± 10.15^{ab}	36.67 ± 15.91^{b}	45.17 ± 22.65^{bc}	28.33 ± 8.89^{bc}	26.17 ± 10.52^{bc}	11.00 ± 4.00^{ab}

Superscript letters denote a statistically significant difference ($p < 0.05$) from the indicated group within the same column. [a] indicates significantly different from Group A; [b] indicates significantly different from Group B; [c] indicates significantly different from Group C; [d] indicates significantly different from Group D.

Table S4. Protective effect of *Withania somnifera* against levamisole [Raw data pertaining to Figure 3(B)]

Group	Treatment	0 h	Day 1	Day 2	Day 3	Day 4	Day 5
A	Worms incubated in M9 buffer	111.11 ± 39.51^{bcd}	110.00 ± 14.10^{b}	85.00 ± 8.14^{c}	76.22 ± 17.51^{bcd}	67.33 ± 13.09^{bcd}	62.78 ± 15.54^{bcd}
B	Levamisole pre-treated worms incubated in M9 buffer	33.11 ± 28.05^{a}	66.00 ± 26.02^{ac}	57.33 ± 26.49^{c}	42.11 ± 16.01^{acd}	17.89 ± 6.83^{acd}	18.78 ± 11.95^{acd}
C	Levamisole pre-treated worms incubated with *W. somnifera*	33.44 ± 16.37^{a}	122.78 ± 38.41^{b}	120.00 ± 31.50^{abd}	116.89 ± 33.52^{ab}	99.56 ± 24.69^{abd}	95.89 ± 15.43^{abd}
D	Levamisole pre-treated worms incubated with dopamine	38.44 ± 13.35^{a}	100.50 ± 40.50	69.00 ± 31.21^{c}	123.89 ± 30.55^{ab}	178.44 ± 40.73^{abc}	370.78 ± 29.56^{abc}

Superscript letters denote a statistically significant difference ($p < 0.05$) from the indicated group within the same column. [a] indicates significantly different from Group A; [b] indicates significantly different from Group B; [c] indicates significantly different from Group C; [d] indicates significantly different from Group D. Shaded cells (▭) indicate presence of progenies, who also contributed towards the measured activity count.

Table S5. Protective effect of Withania somnifera against $MnCl_2$ [Raw data pertaining to Figure 4(A)]

Group	Treatment	0 h	15 min	1 h	3 h	5 h	Day 1	Day 2	Day 3	Day 4	Day 5
A	Worms incubated in M9 buffer (control)	79.00 ± 29.22	114.33 ± 24.11^{bd}	104.00 ± 18.73^{b}	103.67 ± 13.26^{bc}	121.33 ± 12.85^{b}	52.50 ± 18.92^{b}	46.33 ± 10.73^{bc}	29.50 ± 14.29^{b}	23.67 ± 12.24^{b}	13.83 ± 7.99^{b}
B	Worms challenged with $MnCl_2$	91.83 ± 13.57	39.67 ± 20.48^{ac}	59.17 ± 6.68^{acd}	48.00 ± 14.79^{acd}	51.00 ± 6.57^{acd}	1.83 ± 1.83^{acd}	2.67 ± 3.61^{acd}	4.33 ± 4.46^{acd}	2.17 ± 3.25^{ad}	0.17 ± 0.41^{acd}
C	*W. somnifera* pre-treated worms challenged with $MnCl_2$	95.33 ± 18.57	122.00 ± 13.33^{bd}	133.17 ± 18.28^{b}	133.50 ± 17.85^{ab}	129.50 ± 28.91^{b}	24.83 ± 11.53^{bd}	22.33 ± 9.44^{ab}	13.67 ± 3.14^{b}	8.50 ± 3.99^{d}	15.17 ± 5.00^{b}
D	Dopamine pre-treated worms challenged with $MnCl_2$	82.67 ± 23.81	68.00 ± 15.11^{ac}	107.17 ± 8.35^{b}	97.17 ± 23.15^{b}	108.17 ± 33.28^{b}	58.83 ± 18.39^{bc}	36.83 ± 10.76^{b}	25.67 ± 9.37^{b}	29.50 ± 13.63^{bc}	12.17 ± 6.74^{b}

Superscript letters denote a statistically significant difference ($p < 0.05$) from the indicated group within the same column. [a] indicates significantly different from Group A; [b] indicates significantly different from Group B; [c] indicates significantly different from Group C; [d] indicates significantly different from Group D.

Table S6. Protective effect of *Withania somnifera* against $MnCl_2$ [Raw data pertaining to Figure 4(B)]

Group	Treatment	0 h	1 h	Day 1	Day 2	Day 3	Day 4	Day 5
A	Worms incubated in M9 buffer	111.11 ± 39.51^{bcde}	107.44 ± 38.50^{bcde}	110.00 ± 14.10^{bcde}	85.00 ± 8.14^{bde}	76.22 ± 17.51^{bde}	67.33 ± 13.09^{bd}	62.78 ± 15.54^{bde}
B	$MnCl_2$ pre-treated worms incubated in M9 buffer	26.22 ± 21.42^{a}	28.44 ± 20.39^{a}	33.78 ± 21.49^{acde}	26.67 ± 18.92^{ac}	21.00 ± 12.04^{ace}	12.56 ± 8.73^{acde}	12.00 ± 5.77^{acde}
C	$MnCl_2$ pre-treated worms incubated with *W. somnifera*	31.22 ± 20.29^{a}	50.44 ± 11.36^{a}	80.11 ± 24.12^{ab}	89.56 ± 23.72^{bde}	98.11 ± 34.80^{bde}	104.89 ± 33.33^{bd}	98.89 ± 47.39^{bd}
D	$MnCl_2$ pre-treated worms incubated with cortisol	38.56 ± 15.31^{a}	45.22 ± 18.57^{a}	66.00 ± 12.28^{ab}	49.44 ± 14.27^{ac}	39.44 ± 13.51^{ac}	44.00 ± 16.85^{abce}	26.89 ± 7.27^{abce}
E	$MnCl_2$ pre-treated worms incubated with dopamine	28.67 ± 10.25^{a}	43.00 ± 14.48^{a}	73.89 ± 11.25^{ab}	47.56 ± 8.38^{ac}	36.78 ± 6.02^{abc}	79.11 ± 28.54^{bd}	168.22 ± 78.73^{abd}

Superscript letters denote a statistically significant difference ($p < 0.05$) from the indicated group within the same column. [a] indicates significantly different from Group A; [b] indicates significantly different from Group B; [c] indicates significantly different from Group C; [d] indicates significantly different from Group D. [e] indicates significantly different from Group E. Shaded cells (▭) indicate presence of progenies, who also contributed towards the measured activity count.

Table S7. *Withania somnifera* supports *Caenorhabditis elegans* in recovering from heat shock [Raw data pertaining to Figure 5(A)]

Group	Treatment	0 h	1 h	Day 1	Day 2	Day 3	Day 4	Day 5
A	Worms incubated in M9 buffer	111.11 ± 39.50^{bc}	121.66 ± 39.30^{bc}	106.16 ± 15.18^{bc}	85.00 ± 8.13^{bc}	69.16 ± 16.58^{b}	67.33 ± 13.08^{b}	62.77 ± 15.53^{b}
B	Worms exposed to heat	9.33 ± 8.58^{a}	6.66 ± 6.43^{a}	31.00 ± 22.55^{a}	13.55 ± 14.67^{ac}	10.33 ± 8.59^{ac}	5.33 ± 5.80^{ac}	1.77 ± 3.27^{ac}
C	Worms exposed to heat in presence of *W. somnifera*	3.33 ± 6.16^{a}	2.66 ± 5.60^{a}	47.83 ± 14.63^{a}	55.33 ± 12.80^{ab}	44.16 ± 16.06^{b}	86.11 ± 53.45^{b}	103.22 ± 64.93^{b}

Superscript letters denote a statistically significant difference ($p < 0.05$) from the indicated group within the same column. [a] indicates significantly different from Group A; [b] indicates significantly different from Group B; [c] indicates significantly different from Group C. Shaded cells (▭) indicate presence of progenies, who also contributed towards the measured activity count.

Legend to Supplementary Videos

Supplementary videos can be accessed at either of the following links:
https://www.biorxiv.org/content/10.64898/2025.12.18.695325v1.supplementary-material
https://doi.org/10.5281/zenodo.17994417

Videos were captured using a Magnus Camera (5.1 MP) attached to a binocular microscope (Labomed Vision 2000) equipped with halogen light source. While all videos were captured observing through 4X objective, videos I1-I5 were captured using 10X objective.

Assays with chemical stressors

A1-A2: Health control for assays pertaining to the prophylactic potential of WSRE. Gnotobiotic worm in M9 buffer (devoid of plant extract or toxin) captured at day zero (A1) and day 4 (A2). Active movement is visible.

A3-A4: Health control for assays pertaining to therapeutic potential of WSRE, post-toxin exposure. Worms captured on day one (A3). Worms captured on day three (A4).

B1: Worms challenged with rotenone (3 µM) captured after 60 min of toxic shock. **B2**: Extract (600 ppm)-pre-exposed worms challenged with rotenone captured after 60 min of toxic shock. **B3**: Dopamine-pre-exposed worms challenged with rotenone captured after 60 min of toxin shock. Dopamine (5mM) pre-exposure seems to have a positive effect on worm morphology too.

C1: Worms pre-exposed (5 h) to rotenone captured at day two. Most of them have lost activity. **C2:** Rotenone-pre-exposed worms allowed to recover in presence of WSRE captured on day two. **C3**: Rotenone-pre-exposed worms captured at day five. **C4:** Rotenone-pre-exposed worms allowed to recover in presence of WSRE captured on day five. Progeny is also visible. **C5**: Rotenone-pre-exposed worms allowed to recover in presence of dopamine, captured on day five. Vigorous movement, bigger morphology and progeny can be visualized.

D1: Worms challenged with levamisole (100 µM), captured at 5 h post-toxin challenge. **D2:** Extract-pre-exposed worms challenged with levamisole, captured at 5 h post-toxin challenge. **D3**: Worms challenged with levamisole, captured on day-four. Slow movement, and clumping (indication of them being under stress) is visible. **D4**: Extract-pre-fed worms challenged with levamisole, captured on day-four. Clumping is absent, as these worms are able to travel farther from each-other owing to prophylactic protection offered by WSRE, and are under lesser stress. **D5:** Dopamine-pre-fed worms challenged with levamisole, captured on day-four. Degree of clumping is lesser than toxicity control (D3), but more than WSRE-pre-fed worms (D4).

E1: Worms pre-exposed to levamisole, captured on day one. **E2:** Levamisole pre-exposed worms subsequently allowed to recover in presence of WSRE, captured on day one. **E3**: Worms pre-exposed to levamisole, captured on day five. Clumping owing to reduced ability to move farther distances is visible. **E4**: Worms pre-exposed to levamisole, allowed to recover in presence of WSRE, captured on day five. No clumping of worms is observed, as they are able to move farther distances. Progeny worms are also visible. **E5**: Worms pre-exposed to levamisole, allowed to recover in presence of dopamine, captured on day five. Vigorous movement, and many progenies are visible.

F1: Worms challenged with $MnCl_2$ (500 µM) captured 5 h post-toxin challenge. **F2**: Extract-pre-fed worms challenged with $MnCl_2$ captured 5 h post-toxin challenge. **F3:** Worms challenged with $MnCl_2$ captured on day two, have lost all the activity. **F4**: Extract-pre-fed worms challenged with $MnCl_2$, captured on day two. Few live worms are visible. **F5:** Dopamine-pre-fed worms challenged with $MnCl_2$, captured on day two.

G1: $MnCl_2$-pre-exposed (for 3 hour) worms, captured on day three. **G2:** $MnCl_2$-pre-exposed worms allowed to recover in presence of WSRE, captured on day three. Activity is better than health control (**A4**). **G3**: $MnCl_2$-pre-exposed worms captured on day five. **G4**: $MnCl_2$-pre-exposed worms allowed to recover in presence of WSRE, captured on day five. **G5**: $MnCl_2$-pre-exposed worms allowed to recover in presence of dopamine, captured on day five. Vigorous movement, bigger morphology, and progenies can be observed.

Thermorecovery assays

H1: Wild type worms in liquid media captured immediately after a 3 h heat shock at 37°C. Those subject to heat shock in presence of WSRE, not shown here, were also similarly devoid of any movement.; Wild type worms incubated at 22°C in absence **(H2)** or presence **(H3)** of WSRE, allowed to recover from previous heat shock, captured on day two. Better movement in H3 than H2 is visible. Corresponding video for vitamin C is **H4**; On day five, while almost all heat-pre-exposed control worms were dead **(H5),** active movement in wells corresponding to WSRE **(H6)** was visible. Progenies are also visible in **H6**.

Transgenic worms on NGM agar expressing heat-induced paralytic phenotype captured immediately after 4 h heat shock at 35°C, in absence **(I1)** or presence of WSRE **(I2).** Slow movement and stress-induced clumping can be observed in both cases; When worms were allowed to recover from heat shock by incubating at 22°C, at three-hour time point observation, their recovery was better than control **(I3)** in presence of WSRE **(I4)** or caffeine **(I5).** Reduced clumping of worms in I4-I5 is indication of lesser stress.