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1 # Supplementary Information

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3 **β-Carbolines Norharman and Harman change neurobehavior**

4 **causing neurological damage in *Caenorhabditis elegans***

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23 **Text S1. Physiological indicators**

24 Nematodes were maintained at Nematode Growth Medium (NGM medium: 3 g
25 NaCl, 2.5 g tryptone and 17 g agar in 1 L distilled water, using after autoclaving 30
26 min) or K-medium (2.98 g NaCl and 2.386 g KCl in 1 L distilled water, using after
27 autoclaving 30 min) with *Escherichia coli* OP50 as food resources at 20 °C (Strange et
28 al., 2007). The NGM medium on which the nematodes had grown and propagated for
29 several days was cut with a sterilized medicine spoon, and then the cut agar block was
30 pasted on the new NGM medium to propagate the nematodes (Li et al., 2019).

31 A lysis solution (5 % NaClO and 5 M NaOH) was utilized to obtain a synchronized
32 population. To prepare age-synchronized animals, a large number of nematodes in the
33 spawning period were washed down from NGM medium with M9 buffer to precipitate
34 freely, and the excess M9 buffer was absorbed, the lysate was added 1:1, make it fully
35 cracked, centrifuged and washed twice to obtain the precipitated eggs. Washed with
36 M9 buffer repeatedly three times to entirely remove the components of the cracking
37 solution. Finally, the eggs were transferred to NGM medium with fresh OP50 as food,
38 cultured at 20 °C. L1 larval stage was obtained at about 14h after synchronization, and
39 L4 larval stage was obtained at about 48h (Strange et al., 2007).

40

41 **Text S2. Physiological indicators**

42 **Measurements of physiological indicators, including body length and width,**
43 **head thrashes, body bends, and pharyngeal pumping.**

44 The growth of nematodes was evaluated by body length and body width. After
45 exposure, *C. elegans* were transferred to slides and the length and width were measured
46 under the stereomicroscope equipped with a camera (Zeiss stemi305) after being killed
47 by heat (20 worms/treatment) (Zhou et al., 2016). Locomotion behaviors were assessed
48 by head thrashes and body bends. A head thrash is defined as a change in the direction
49 of bending at the middle body. Assuming the nematodes only moved along the x-axis,
50 a body bend is regarded as a change in the direction of nematodes corresponding to the
51 posterior bulb of the pharynx along the y-axis. To assay head thrashes and body bends,
52 the test nematodes were rinsed thoroughly with M9 buffer and transferred to another
53 NGM plate without OP50. Allow its behavior to stabilize for 1 min, and then place it
54 under a stereoscopic microscope to observe the number of head swings and body bends
55 after its behavior recovers. head trashes were counted for 1 min and body bends were
56 counted for the 20s with the stereomicroscope by eye. Each behavioral analysis
57 examined fifteen nematodes per treatment (Wu et al., 2013).

58 The pharyngeal pumping rate was determined as previously described (Liu et al.,
59 2013), nematodes after the end of exposure were collected, and the tested nematodes
60 were washed three times with sterile K buffer, put on OP50 coated medium and then
61 incubated at 20°C for 2h to enter a stable fed state. The pharyngeal pumping frequency
62 (the beating of the nematode's pharynx back and forth scored as a pumping pharynx

63 once) within the 20s was observed and recorded using the stereomicroscope, fifteen
64 nematodes per treatment were examined.

65 **Text S3. Foraging behavior**

66 Before the test, *E. coli* OP50 was cultivated within a 0.5 cm radius from the center
67 in a 9 cm agar medium, and 1 μl of 60 μM levamisole solution, which played an
68 anesthetic effect, was applied to the OP50 colony. Next, the exposed nematodes were
69 washed three times with M9 buffer, and about 40 nematodes were transferred to the
70 starting position (4 cm away from the *E. coli* center) of a test Petri dish (**Fig. 2A**). Each
71 test was replicated 4 times. After 2, 4, 8, and 24 hours of incubation at 20°C, nematode
72 numbers in OP50 exposed colonies were counted in each plate using the
73 stereomicroscope. The foraging ability of *C. elegans* was derived by counting the
74 percentage of the number of *C. elegans* in contact with the food source to the total
75 number of *C. elegans* in the test Petri dish.

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Text S4. Chemotaxis assay

Before the test, prepare the Petri dishes: A typical 9 cm Petri dish was used for each experimental group to evaluate chemotaxis behavior, and a piece of NaCl agar block (100 mM) was placed 3 cm from the center (**Fig. 2B point N**), and the corresponding opposite position was set as control (without NaCl) (**Fig. 2B point C**). The Petri dishes put in the agar block were stored overnight at 4 °C to obtain the concentration gradient of NaCl. Fifteen minutes before the assay, the NaCl agar was removed and 1 μL levamisole solution was applied to each of the two test points. Approximately 50 nematodes were placed at the starting position (**Fig. 2B point S**).

The chemotaxis index (CI) was obtained using the formula: (the number of *C. elegans* in the NaCl-containing part/the number in the NaCl-free part)/the total number of *C. elegans* on test Petri dishes). A positive CI indicated an attraction to the salt, and a negative CI indicated an aversion, as described in the literature. Every concentration was replicated four times (Wu et al., 2015).

92 **Text S5. Determination of ROS accumulation, SOD, CAT activity and MDA**
93 **content**

94 Experimental worms were washed three times with M9 buffer and moved into 24-
95 well plates that contained 50 μM of CM-H_2DCFDA for 1 h at 20 °C, and then worms
96 were washed with M9 buffer and anesthetized by 1 μL 60 μM levamisole solution and
97 transferred onto a glass slide. The fluorescence images were obtained by laser confocal
98 fluorescence microscope (Zeiss LSM800) under the excitation at 485 nm. For each
99 group, 15 nematodes were photographed. Fluorescence intensity was analyzed with
100 ImageJ.

101 After exposure, the nematodes were harvested and washed with M9 buffer, then
102 transferred into Ep tubes. The suspension was homogenized by vortex grinding,
103 followed by centrifugation at 8000 rpm for 10 min at 4 °C. The supernatant was
104 collected to determine the total protein, the total superoxide dismutase (SOD) and
105 catalase (CAT) activities and the content of malondialdehyde (MDA) according to the
106 kit instructions, respectively. The SOD, CAT and content of MDA were normalized by
107 protein content and represented as U/mg prot, U/mg prot and mg prot/ml, respectively.
108 Each indicator was repeated three times for each concentration. SOD, CAT activities
109 and the content of MDA in all tissues were measured using the assay kit (Nanjing Jian
110 Cheng Bioengineering Institute), the total protein assay kit (BCA method) (Nanjing
111 Jian Cheng Bioengineering Institute).
112

Text S6. qRT-PCR method

The RNA of nematodes was extracted by TRNzol (Tiangen, Beijing, China) and reverse-transcribed to obtain cDNA using FastKing gDNA Dispelling RT SuperMix (Tiangen, Beijing, China). The expression of tested genes was examined using MyGo Pro (IT-IS Life Science) with a SuperReal Premix Plus (SYBR Green) kit (Tiangen, Beijing, China). Gene expression was analyzed by the $2^{-\triangle\triangle Ct}$ method and actin-1 mRNA was used to normalize the expression level. For the analysis of each gene, three replicates at least were measured per concentration. The designed primers were shown in **Table S1**.

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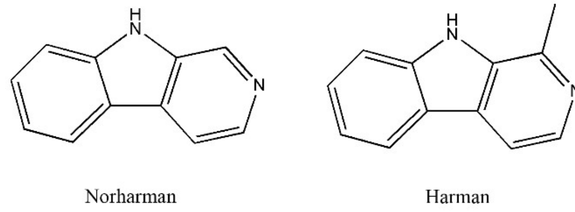

124 **Fig. S1**. The chemical structures of Norharman and Harman

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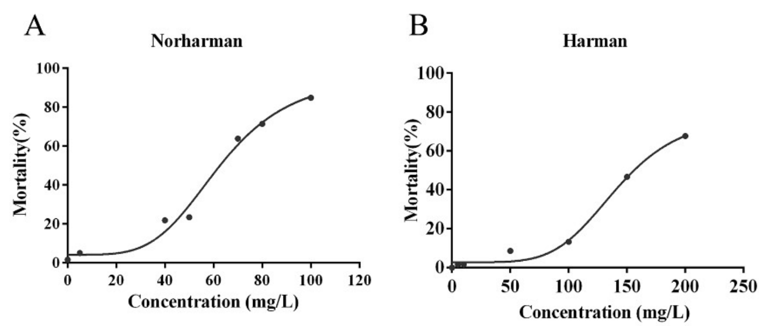

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127 **Fig. S2.** Dosage-mortality curve after acute exposure. (A) Norharman. (B) Harman.

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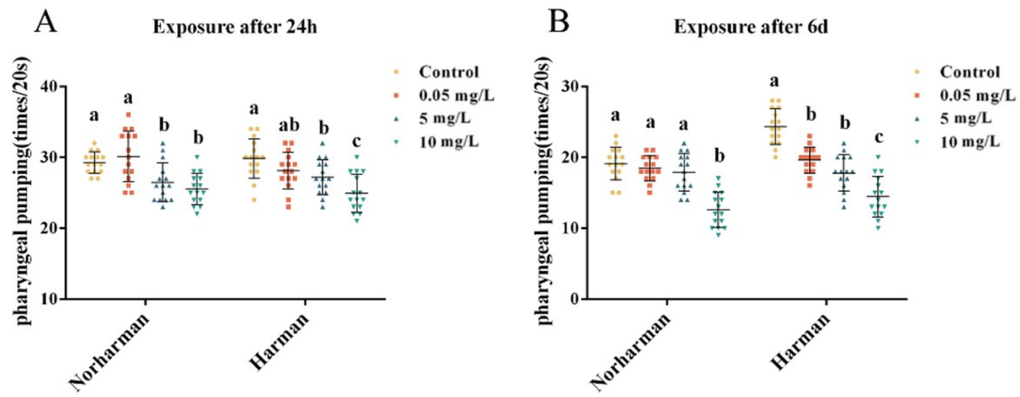

134 **Fig. S3.** Effects on *C. elegans* pharyngeal pumping frequency after exposure. (A) after 24h exposure

135 of Norharman and Harman. (B) after 6d exposure to Norharman and Harman. Data are presented as

136 the mean ± SD. Significance ($p < 0.05$) among groups is denoted by different letters.

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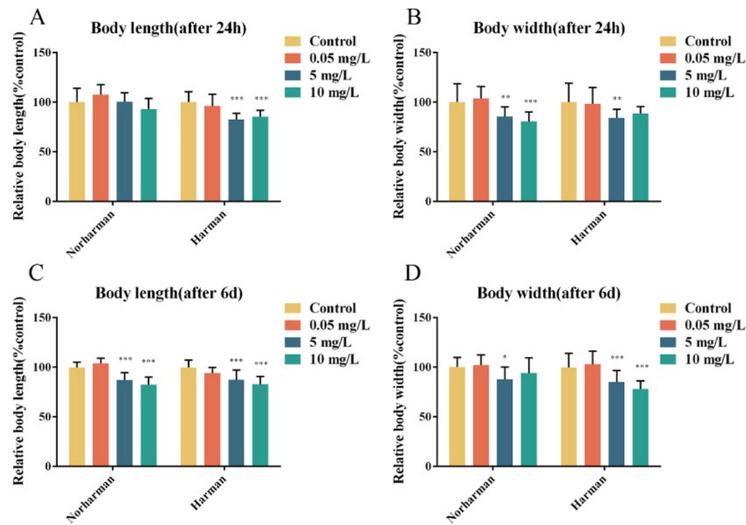


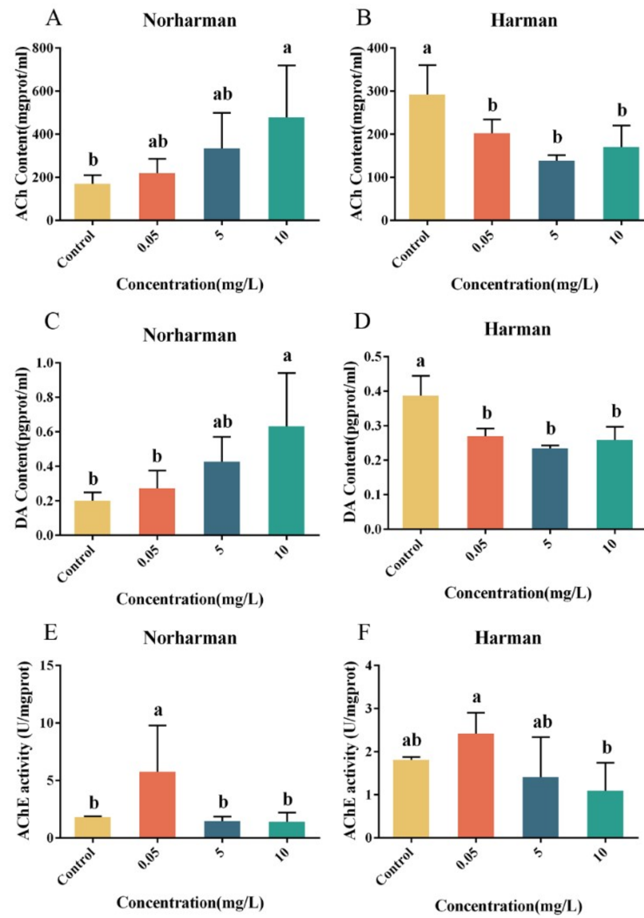
138 **Fig. S4.** Effects on *C. elegans* body length and body width after exposure to Norharman and

139 Harman. (A) The body length of the nematodes after 24h. (B) The body width of the nematodes

140 after 24h. (C) The body length of the nematodes after 6d. (D) The body width of the nematodes

141 after 6d. Each group randomly selected 20 worms for measurement. Data are presented as the

142 mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001.



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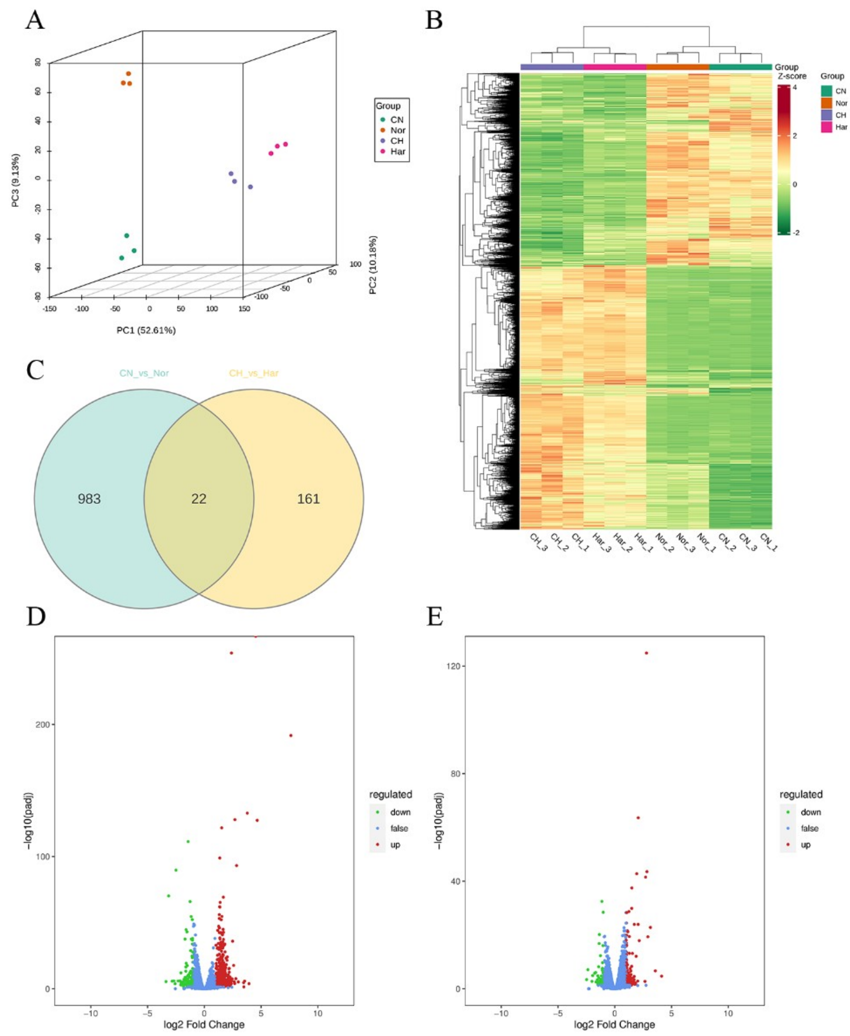
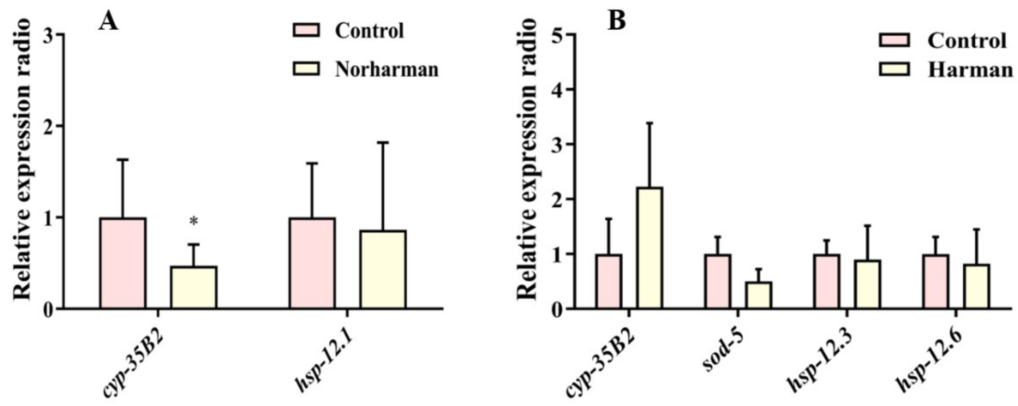
144 **Fig. S5.** Effects on the content of ACh, DA and AChE activity after exposure. (A, B)
145 the content of ACh in *C. elegans* after exposure to Norharman and Harman. (C, D) the
146 content of DA in *C. elegans* after exposure to Norharman and Harman. (E, F) AChE
147 activity in *C. elegans* after exposure to Norharman and Harman. Data are presented as
148 the mean ± SD. Significance ($p < 0.05$) among groups is denoted by different letters.



149 **Fig. S6.** RNA-Seq analysis results. (A) principal component analysis (PCA). (B) 150 Differential gene clustering heat map. (C) Venn diagram of the differential genes in 151 different groups. (D) Differential gene volcanic map of exposure of Norharman. (E) 152 Differential gene volcanic map of exposure of Harman. (Nor and Har represent 153 Norharman and Harman groups, respectively, CN represents the control group for Nor, 154 and CH represents the control group for Har).



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156 **Fig. S7.** Validation of gene expression patterns using qRT-PCR.

157 **Table S1. Primer for qRT-PCR**

Primer		Primer sequence (5’-3’)
Actin-1	fw	AGAAGAGCACCCAGTCCTCC
	re	GAAGCGTAGAGGGAGAGGAC
cyp-35B2	fw	CGATTTCTTTGACCTTGCCGTTGG
	re	AGTGAATAAGTCTGAAGCCGAGTCC
hsp-12.1	fw	ATTACAACTGACTCGGCGGCTTC
	re	GGTCCAAAGAATCCAGCATCAAGTC
sod-5	fw	TGCCAATGCCGTTCTTCCACAG
	re	AACAGTTCCGAAGACAGCAGTTCC
hsp-12.3	fw	GGCTCACACAACCAAAGACGATAAG
	re	TTCCGCTGCCATCCAACTTGC
hsp-12.6	fw	CAGTGATGGCTGACGAAGGAACC
	re	TGGGAGGAAGTTATGGGCTTCTAGG

159 Reference:

160 Li, Y., Chu, Q., Liu, Y., Ye, X., Jiang, Y., Zheng, X., 2019. Radix Tetrastigma
161 flavonoid ameliorates inflammation and prolongs the lifespan of Caenorhabditis
162 elegans through JNK, p38 and Nrf2 pathways. Free Radical Research 53, 562-
163 573.

164 Liu, S., Saul, N., Pan, B., Menzel, R., Steinberg, C.E., 2013. The non-target organism
165 Caenorhabditis elegans withstands the impact of sulfamethoxazole.
166 Chemosphere 93, 2373-2380.

167 Strange, K., Christensen, M., Morrison, R., 2007. Primary culture of Caenorhabditis
168 elegans developing embryo cells for electrophysiological, cell biological and
169 molecular studies. Nat Protoc 2, 1003-1012.

170 Wu, Q., Nouara, A., Li, Y., Zhang, M., Wang, W., Tang, M., Ye, B., Ding, J., Wang,
171 D., 2013. Comparison of toxicities from three metal oxide nanoparticles at
172 environmental relevant concentrations in nematode Caenorhabditis elegans.
173 Chemosphere 90, 1123-1131.

174 Wu, T., He, K., Zhan, Q., Ang, S., Ying, J., Zhang, S., Zhang, T., Xue, Y., Tang, M.,
175 2015. MPA-capped CdTe quantum dots exposure causes neurotoxic effects in
176 nematode Caenorhabditis elegans by affecting the transporters and receptors of
177 glutamate, serotonin and dopamine at the genetic level, or by increasing ROS,
178 or both. Nanoscale 7, 20460-20473.

179 Zhou, D., Yang, J., Li, H., Cui, C., Yu, Y., Liu, Y., Lin, K., 2016. The chronic toxicity
180 of bisphenol A to Caenorhabditis elegans after long-term exposure at

181 environmentally relevant concentrations. Chemosphere 154, 546-551.