Supplementary data

Neuroprotective and memory-enhancing effects of antioxidant peptide from walnut (*Juglans regia* L.) protein hydrolysates

Mingchuan Liu^a, Shengjie Yang^a, Jinping Yang^a, Yita Lee^c, Junping Kou^{b**}, and Chaojin Wang^{a,c*}

^a R&D Center, Sinphar Tian-Li Pharmaceutical Co., Ltd., Hangzhou 31100, China

^b Department of Complex Prescription of TCM, China Pharmaceutical University, Nanjing 211198,

China

^c R&D Center, Sinphar Pharmaceutical Co., Ltd., Ilan (Taiwan) 269, Republic of China

[¶]Both authors contributed equally to this work.

*Corresponding author. Sinphar Tian-Li Pharmaceutical Co., Ltd., Yuhang Economic & Technological Development Zone, Hangzhou, China 311100. Tel.: +86 571 8616 8933; Fax: +86 571 8616 8991.

E-mail address: wangzr1960@126.com

**Corresponding author. Department of Complex Prescription of TCM, China Pharmaceutical University, Jiangning St., Nanjing, China 211198. Tel.: +86 25 8618 5158; Fax: +86 25 8618 5158.

E-mail address: koujp1966@126.com

Table 1S. Effect of WP on the target time and crossing times of scopolamine-induced acquisition impairment in the Morris water maze test.

| Groups | Dose (mg/kg) | Target-time (s) | Crossing-times (N) |
|------------------|--------------|-----------------------|--------------------|
| Normal control | - | 34.2 ± 6.1 | 2.4 ± 0.5 |
| Negative control | - | $25.8 \pm 2.3^{\#\#}$ | $1.7\pm0.7^{\#}$ |
| Nmodipine | 30 | 29.4 ± 8.2 | $3.8 \pm 1.9^{**}$ |
| Low-dose WP | 30 | $30.3 \pm 4.9^*$ | 2.8 ± 1.6 |
| Medium-dose WP | 100 | $29.1 \pm 3.9^*$ | $2.8 \pm 1.2^*$ |
| High-dose WP | 300 | 27.7 ± 5.9 | $4.7 \pm 1.5^{**}$ |

^{**} P < 0.05 compared with normal control group, *** P < 0.01 compared with normal control group, ** P < 0.05 compared with negative control group, *** P < 0.01 compared with negative control group.

Table 2S. Effect of WP on the escape latency of sodium nitrite-induced consolidation impairment in the Morris water maze test.

| Groups | Dose — (mg/kg) | Escape latency (s) | |
|------------------|----------------|---------------------|--------------------|
| | | Before injection of | After injection of |
| | | sodium nitrite | sodium nitrite |
| Normal control | - | 14.4 ± 10.5 | 12.0 ± 14.1 |
| Negative control | - | 21.3 ± 20.3 | 27.6 ± 34.8 |
| Nmodipine | 30 | 20.8 ± 13.3 | 18.9 ± 14.9 |
| Low-dose WP | 30 | 18.1 ± 14.4 | 8.1 ± 11.7 |
| Medium-dose WP | 100 | 12.0 ± 4.7 | 10.8 ± 6.4 |
| High-dose WP | 300 | 13.3 ± 11.2 | 4.7 ± 4.2 |

Table 3S. Effect of WP on the target time and crossing times of ethanol-induced reproduction impairment in the Morris water maze test.

| Groups | Dose (mg/kg) | Target-time (s) | Crossing-times (N) |
|------------------|--------------|-----------------|--------------------|
| Normal control | - | 35.1 ± 5.7 | 3.5 ± 2.3 |
| Negative control | - | 26.2 ± 4.5## | $0.9 \pm 1.0^{\#}$ |

| Nmodipine | 30 | 35.0 ± 8.9 | 1.7 ± 1.7 |
|----------------|-----|----------------|-----------------|
| Low-dose WP | 30 | 30.8 ± 9.5 | 0.9 ± 1.4 |
| Medium-dose WP | 100 | 25.4 ± 5.8 | 0.8 ± 0.6 |
| High-dose WP | 300 | $32.2\pm7.4^*$ | $3.8 \pm 4.2^*$ |

^{**} P < 0.01 compared with normal control group, * P < 0.05 compared with negative control group.

Table 4S. Effect of WP on sodium nitrite-induced consolidation impairment in the step-down test.

| Groups | Dose (mg/kg) | Step-down latency (s) | Error times (<i>N</i>) |
|------------------|--------------|-----------------------|--------------------------|
| Normal control | - | 221.2 ± 70.6 | 1.0 ± 0.95 |
| Negative control | - | $94.7 \pm 49.3^{##}$ | 3.6 ± 2.5 ## |
| Nmodipine | 30 | 237.7 ± 79.7** | 1.0 ± 1.2** |
| Low-dose WP | 30 | 252.8 ± 72.7** | $0.4 \pm 0.6^{**}$ |
| Medium-dose WP | 100 | $273.2 \pm 43.7^{**}$ | $0.7 \pm 1.5^{**}$ |
| High-dose WP | 300 | $240.7 \pm 66.8^{**}$ | $0.8 \pm 1.0^{**}$ |

^{***} P < 0.01 compared with normal control group, *** P < 0.01 compared with negative control group.

Table5S. Effect of WP on ethanol-induced reproduction impairment in the step-down test.

| Groups | Dose (mg/kg) | Step-down latency (s) | Error times (N) |
|------------------|--------------|-----------------------|--------------------|
| Normal control | - | 212.0 ± 84.2 | 0.8 ± 0.9 |
| Negative control | - | 46.3 ± 26.5 ## | 5.7 ± 2.9## |
| Nmodipine | 30 | 99.6 ± 98.7 | $2.5 \pm 1.4^*$ |
| Low-dose WP | 30 | 199.3 ± 75.5** | $2.0 \pm 2.0^{**}$ |
| Medium-dose WP | 100 | 142.1 ± 113.5** | $2.8 \pm 2.2^*$ |
| High-dose WP | 300 | $146.1 \pm 73.8^{**}$ | 3.2 ± 3.4 |

^{***} P < 0.01 compared with normal control group, ** P < 0.05 compared with negative control group, ** P < 0.01 compared with negative control group.