Possible molecular mechanism for acute encephalopathy by angel-wing mushroom ingestion : Involvement of three constituents in onset

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# Possible molecular mechanism for acute encephalopathy by angel-wing mushroom ingestion – involvement of three constituents in onset –

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## M 1 2 3 4 5 6 7 8 9 10 11



## Figure S1.

**SDS-PAGE following protease assay using BSA and IgG as a substrate.** Lane 1: PPL, lane 2: PC, lane 3: BSA, lane 4: IgG, lane 5: PPL and BSA, lane 6: PPL and IgG, lane 7: complex (PC and PPL), lane 8: PC and BSA, lane 9: PC and IgG, lane 10: complex (PC and PPL) and BSA, lane 11: complex (PC and PPL) and IgG, M: molecular mass standards (XL-Ladder Low).



#### Figure S2.

MALDI-TOF-MS analysis after protease assay of complex (PC and PPL) using insulin as substrate. PC, PPL and insulin were mixed, and then incubated at  $37^{\circ}$ C for 1, 3, 6, 9, 12, 24, 48, and 72 hours. The red arrow indicated the molecular ion of insulin at m/z 5802.



### Figure S3.

MALDI-TOF-MS analysis following protease assay using insulin as a substrate. Control: insulin (0.25 mg/ml), PC: PC (0.75 mg/ml), PPL: PPL (0.75 mg/ml, complex: PC (0.75 mg/ml) and PPL (0.75 mg/ml). The mixtures including substrate (insulin) were incubated at 37°C for 72 hours. The red arrow indicated the molecular ion of insulin at m/z 5802.



## Figure S4.

**Immunohistochemistry for ssDNA in the hippocampus of mouse brain.** The black arrows indicate ssDNA-immunopositive cells. Control : 10 mM PBS, PC: PC (24 mg/kg BW), PPL: PPL (30 mg/kg BW), PA: PA (70 mg/kg BW), PC + PPL: PC (24 mg/kg BW) and PPL (30 mg/kg BW), PC + PPL + PA: PC(24 mg/kg BW), PPL(30 mg/kg BW) and PA (70 mg/kg BW).



Expansion view of control (PBS) in Figure. S4.



Expansion view of PC in Figure S4.



Expansion view of PPL in Figure S4.



Expansion view of PA in Figure S4.



Expansion view of PC + PPL in Figure S4.



Expansion view of PC + PPL + PA in Figure S4.



## Figure S5.

**Immunohistochemistry for anti His-Tag antibody in the hippocampus of mouse brains other than that shown in Figure 4B.** Control: 10 mM PBS, rPPL: rPPL (30 mg/kg BW), PC + PPL: PC (24 mg/kg BW) and rPPL (30 mg/kg BW).



Figure S6.

**Immunohistochemistry for anti His-Tag antibody in the hypothalamus of mouse brains other than that shown in Figure 4C.** Control: 10 mM PBS, rPPL: rPPL (30 mg/kg BW), PC + PPL: PC (24 mg/kg BW) and rPPL (30 mg/kg BW).



## Figure S7.

**Immunohistochemistry for NeuN in the hippocampus of mouse brain.** Control: 10 mM PBS, PC: PC (24 mg/kg BW), PPL: PPL (30 mg/kg BW), PA: PA (70 mg/kg BW), PC + PPL: PC (24 mg/kg BW) and PPL (30 mg/kg BW), PC + PPL + PA: PC (24 mg/kg BW), PPL (30 mg/kg BW) and PA (70 mg/kg BW).



### Figure S8.

**Immunohistochemistry for MAP2 in the hippocampus of mouse brain.** Control: 10 mM PBS, PC: PC (24 mg/kg BW), PPL: PPL (30 mg/kg BW), PA: PA (70 mg/kg BW), PC + PPL: PC (24 mg/kg BW) and PPL (30 mg/kg BW), PC + PPL + PA: PC (24 mg/kg BW), PPL (30 mg/kg BW) and PA (70 mg/kg BW).



### Figure S9.

**Immunohistochemistry for GFAP in the hippocampus of mouse brain.** Control: 10 mM PBS, PC: PC (24 mg/kg BW), PPL: PPL (30 mg/kg BW), PA: PA (70 mg/kg BW), PC + PPL: PC (24 mg/kg BW) and PPL (30 mg/kg BW), PC + PPL + PA: PC (24 mg/kg BW), PPL (30 mg/kg BW) and PA (70 mg/kg BW).



## Figure S10.

**Immunohistochemistry for Glut1 in the hippocampus of mouse brain.** Control: 10 mM PBS, PC: PC (24 mg/kg BW), PPL: PPL (30 mg/kg BW), PA: PA (70 mg/kg BW), PC + PPL: PC (24 mg/kg BW) and PPL (30 mg/kg BW), PC + PPL + PA: PC (24 mg/kg BW), PPL (30 mg/kg BW) and PA (70 mg/kg BW).



## Figure S11.

**SDS-PAGE following protease assay using BSA as a substrate.** Lane 1: PC, lane 2: rPPL, lane 3: BSA, lane 4: PC and rPPL, lane 5: PC and BSA, lane 6: rPPL and BSA, lane 7: complex (PC and rPPL) and BSA, lane M: molecular mass standards (XL-Ladder Low).