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Ultrapure and potent tannic acid (UPPTA) is a novel inhibitor of D-amino acid oxidase to improve the *N*-methyl-D-aspartate function of CNS disorders

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ABSTRACT

Tannic acids (TAs), a group of polyphenols, have been used as a traditional Chinese medicine for millennia. Nevertheless, due to the complexity of TAs' compositions, no studies have specifically focused on the relationship between the compositions and their biological activities. In this study, by applying HPLC-PDA-MS and NMR analyses of the compositions and a D-amino acid oxidase (DAAO) assay for biological activities, we discovered that the number of galloyl moieties was positively correlated with the inhibitory potency of DAAO. Furthermore, the ultrapure and potent tannic acid (UPPTA) that we prepared by removing impurities and low-galloyl-moiety TAs exhibited the strongest DAAO inhibitory potency compared with all the commercial TAs. The *in vivo* studies using MK-801 treated mice showed that the UPPTA improved the *N*-methyl-D-aspartate (NMDA)-hypofunction-induced hyperactivity, working memory, and sensory motor gating deficits. In sum, the UPPTA demonstrated superior central nervous system (CNS) responses suggesting its potential as a novel therapeutic candidate for treating CNS disorders through DAAO inhibition.

Introduction

Tannic acids (TAs), enriched in different species of gallnut worldwide, have been applied in food and medicine for centuries. In China, Chinese gallnut (also called Wu-bei-zi) with abundant TAs has been used in herbal recipe for the treatments of diarrhea, bleeding, coughing, and detoxification for more than two thousand years (Tian et al., 2009; Ren et al., 2021). In India, some Unani medicine containing tannic acids such as Barhami (*Bacopa monnieri*), a traditional treatment of dementia (Fatima et al., 1997). In Western countries, TAs had been used as an antidote to soak up poisons from late 19th to early 20th centuries (Daly and Cooney, 1978). During World War I, TAs were used as a dressing to treat severe burn injuries, which significantly reduced mortality rates (Chokotho and van Hasselt, 2005). At present, TAs are used as a direct food additive in numerous food and beverage products because of their well-known safety (Reker et al., 2020). Attributed to their astringent attribute, TAs have been used as additives in medicinal products for humans, for instance in the drug product for treating diarrhea (Loeb et al., 1989). In addition, TAs have the potential to be used as drugs for improving diabetes and are beneficial to human health, including accelerating blood clotting, reducing blood pressure, and regulating immune responses (Chung et al., 1998; Esmaie et al., 2019).

Commercial TAs are of various origins, and the crude extract is isolated from either sumac (*Rhus semialata*) galls (Chinese gallotannin), Aleppo oak (*Quercus infectoria*) galls (Trukish gallotannin), or sumac (*R. coriaria* or *R. typhina*) leaves (sumac gallotannin). The structures of TAs are polygalloyl glucose esters composed of 2 to 12 units of galloyl moieties per molecule that complicates their composition in nature (Fig. 1). Although commercial suppliers provide a nominal molecular weight for TAs (1701 g/mol), they are mixtures of gallotannins with compositions poorly understood and defined.

N-methyl-D-aspartate receptor-mediated neurotransmission (NMDARMN) serves as the molecular engine of learning, memory, and cognition, which are key areas that regulate the manifestation of central nervous system (CNS) disorders (Tsai, 2016). NMDAR is a glutamate receptor whose activation requires two distinct agonists: glutamate and aspartate; in addition, two amino acids, D-serine or glycine, are obligatory co-agonists. D-amino acid oxidase (DAAO) is a flavoenzyme of peroxisomes responsible for degrading D-serine (Verrall et al., 2010). Therefore, inhibition of DAAO would augment D−serine levels, leading to NMDAR function enhancement. As such, DAAO has been targeted for treating CNS disorders associated with D-serine and/or glutamatergic neurotransmission (Tsai, 2016).

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Abbreviations

ANOVA	analysis of variance;
CNS	central nervous system;
DAAO	D-amino acid oxidase;
ddH_2O	deionized water;
ESI	electrospray ionization;
fAβ	β-amyloid fibrils;
FAD	flavin adenine dinucleotide disodium salt hydrate;
G	galloyl group of tannic acids;
HRP	horseradish peroxidase;
i.p.	intraperitoneal;
LD_{50}	median lethal dose;
min	minute;
MK-801	(+) MK-801 hydrogen maleate;
MTD	maximum tolerated dose;
NMDA	*N*-methyl-D-aspartate;
NMDAR	*N*-methyl-D-aspartate receptor;
NMDARMN	*N*-methyl-D-aspartate receptor-mediated neurotransmission;
NOAEL	non-observable-adverse-effect level;
NS	non-stimulus;
OPD	o-phenylenediamine;
PA	pulse alone;
PDA	photodiode array;
p.o.	oral gavage;
pp	pre-pulse;
PPI	pre-pulse inhibition;
SD	standard deviation;
sec	second;
S.E.M.	standard error of the mean;
TA	tannic acid;
UPPTA	ultrapure and potent tannic acid

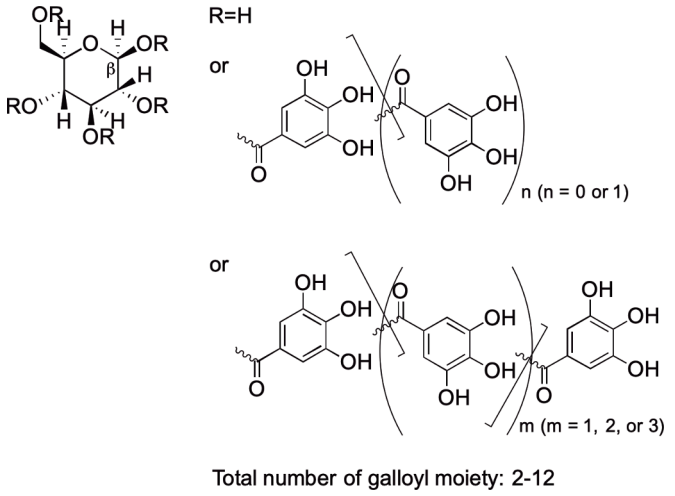

Fig. 1. Chemical structure of natural TAs. TAs consist of a glucose core bonding galloyl moieties through ester bonds. The number of galloyl moieties can be 2 to 12. The number of n or m is the number of galloyl moieties.

In this research, we aimed to understand the structure-activity relationship of each TAs' composition and then applied that to study the differences among in-house purified ultrapure and potent tannic acids (UPPTA) and several commercial TAs in DAAO inhibition. In animal studies, the MTD study was conducted, followed by assessing their effects on hyper-locomotion, pre-pulse inhibition, and working memory in MK-801-induced mice to investigate the effects of TAs on regulating CNS activities.

Materials and methods

Chemicals

Porcine kidney-sourced DAAO, o-phenylenediamine (OPD), horseradish peroxidase (HRP), D-proline, flavin adenine dinucleotide disodium salt hydrate (FAD), phosphate-buffered saline, DMSO, (+) MK-801 hydrogen maleate (MK-801), and formic acid (> 98% purity) were purchased from Sigma-Aldrich. HPLC-grade acetonitrile and methanol were obtained from Merck Millipore. Deionized water (ddH_2O, 18.2 MΩ cm) was prepared using a Barnstead Smart2Pure water purification system (Thermo Scientific). The commercial TAs were purchased from Merck (pharmaceutical grade, Merck KGaA) and Sigma Aldrich (ACS grade, Sigma-Aldrich).

UPPTA preparation

The UPPTA, the purified TAs, was prepared by SyneuRx (pharmaceutical grade, SyneuRx International (Taiwan) Corp.). The crude TAs originated from Chinese gallnuts (*Rhus chinensis*) were subjected to a proprietary processing to acquire the UPPTA.

Isolation of gallotannins from Merck's TAs

A powder of Merck's TAs was dissolved in 1% aqueous acetonitrile solution, separated by a LiChroprep RP-18 column (Lobar Fertigsäule, 40–63 μm, 25 × 310 mm, Merck KGaA, Dramstadt), and fractionated by a multi-step linear solvent gradient of acetonitrile and distilled water, to yield 26 fractions. Each TA fraction with different numbers of galloyl moieties was identified by HPLC-PDA-MS and NMR.

Determination of TAs compositions by HPLC-PDA-MS analysis

The degrees of galloylation of commercial TAs were investigated by HPLC-PDA-MS analysis. The chromatographic analysis was performed by means of a liquid chromatography equipment Shimadzu Prominence with a mass spectrometer LCMS-2020. Separation of TAs was carried out using a Kinetex XB-C18 column (5 μm, 150 × 4.6 mm I.D., Phenomenex), running with linear gradient elution of 1% (v/v) formic acid in water and methanol/acetonitrile (2:8, v/v). The flow rate was at 0.6 mL/min. Scan range of MS was set to 100 to 2000 (m/z) with a scan speed at 10,000 u/s and event time of 0.2 s under negative mode. The Disolvation Line temperature was set at 250 °C with an interface temperature of 350 °C and a heat block of 400 °C. The nebulizing gas flow was set to 1.5 L/min and dry gas flow to 20 L/min.

Animals

All C57BL/6 male mice and related procedures used in this study were approved by the Institutional Animal Care and Use Committee (IACUC) at SyneuRx International (Taiwan) Corp. (IACUC number: SR106002), of which procedures followed the guidelines of NIH on laboratory animal welfare. The mice were purchased from National Laboratory Animal Center (Taiwan). The body weights and ages of study mice were 15–23 g and 8–9 weeks, respectively. C57BL/6 J male mice were grouped, housed (3–5 mice per cage) with food and water available ad libitum in polysulfone ventilated cages (Alternative Design). The

colony was maintained on a 12/12-h light/dark cycle at 22 ± 2 °C, and all behavioral studies were performed during the dark cycle.

The DAAO inhibition assay

The activity of DAAO was determined in vitro by measuring the inhibition of the catabolism of known substrate, D-proline. The cofactor FAD (40 μM) was added to the DAAO stock solution first. For the assay, TAs were mixed with the reaction mixture containing phosphate buffered saline (137 mM NaCl, 3 mM KCl, 10 mM Na_2HPO_4, 2 mM NaH_2PO_4, pH 7.4), HRP (5 U/mL), OPD (0.03%) and 0.6 μg DAAO, and the mixed solution was incubated for about 5 min. After the pre-incubation period, 40 mM D-proline were added as a substrate, and the reaction continued for 10 min. OPD was oxidized to form 2,3-diaminophenazine by HRP. All enzymatic assays were conducted at room temperature. The absorbance wavelength of 2,3-diaminophenazine was measured at 453 nm. The assay was completed in serial dilutions of the inhibitors to generate the IC_{50} and analyzed by Prism (Graphpad Software).

The maximal tolerated dose (MTD) study for the TAs

Sigma's TAs and the UPPTA were subjected to the MTD study on C57BL/6 mice, followed by a 7-day observation period. For Sigma's TAs, mice were randomly assigned into four groups, 3 mice per group: (1) control, (2) 500 mg/kg, (3) 750 mg/kg, and (4) 1000 mg/kg; while for the UPPTA, the mice were randomly assigned into 5 groups, 3 mice per group: (1) control, (2) 3000 mg/kg, (3) 3500 mg/kg, (4) 4000 mg/kg, and (5) 4500 mg/kg. All mice were administered p.o. with the TAs. The animals were observed twice daily or as needed for the signs of mortality, morbidity, respiration, secretion, feces, and water and food intake. The body weight of each mouse, served as an index of physical development and metabolism, was recorded daily throughout the study.

Open field test of TAs in MK-801 treated mice

Open field test is a common measurement of novelty-induced exploratory behavior and general activity in rodents. Mice were randomly assigned into four groups, 5–8 mice per group: (1) saline control, (2) MK-801, (3) Merck's TAs (50 mg/kg) + MK-801, and (4) UPPTA (50 mg/kg) + MK-801. The mice in Groups 2–4 received an acute administration of MK-801 dissolved in normal saline, 0.2 mg/kg via i.p. injection 20 min prior to the behavioral test. The mice were placed in a PLEXIGLAS cage (37.5 × 21.5 × 18 cm) under 50–65 Lux light intensity. Their spontaneous locomotor activities were measured for 30 min using the SMART video tracking system (Panlab, Harvard Apparatus). The travel distance of each mouse was measured as an index of locomotion.

Barnes maze task of TAs in MK-801 treated mice

Barnes maze is a task to evaluate the cognitive function in mice, especially the spatial learning and memory (Rosenfeld and Ferguson, 2014). Mice were randomly assigned into four groups, 6–8 mice per group: (1) saline control, (2) MK-801, (3) Merck TAs (50 mg/kg) + MK-801, and (4) UPPTA (50 mg/kg) + MK-801. The mice in Groups 2–4 received an acute administration of MK-801 dissolved in normal saline, 0.2 mg/kg by i.p. injection 20 min prior to the behavioral test.

The testing apparatus was an elevated (50 cm above the floor) circular PLEXIGLAS plate (100 cm in diameter) with 20 holes (7 cm in diameter, 7 cm between holes) evenly spaced around the perimeter. The mice were trained on the plate to identify an escape box (25 × 8 × 6 cm) hidden beneath the target hole, which was designated as an analog to the hidden platform in the Morris water maze task. Mice were initially placed at the center of the plate covered by an opaque cylinder, which was removed 10 s after the beginning of the trial with both an aversive tone (440 Hz, 85 dB) and light (100 Lux) switched on. The mice were trained to locate the target hole with surrounding four visual cues and escaped from the aversive tone for three training trials per day over 3 consecutive days. The spatial memory was measured by the "probe test". All the training trials and the probe trials were videotaped for 3 min. Then, the escape latency for the training trials and the percentage of time in different quadrants (target, left, right, and opposite) during the probe test were analyzed.

The pre-pulse inhibition test of TAs in MK-801 treated mice

Pre-pulse inhibition (PPI) was used as an index of sensorimotor gating function using SR-LAB startle apparatus (San Diego Instruments). Under 65 dB background noise, each session was composed of 5 min accumulation period followed by 64 trials in four blocks. The pulse alone (PA) trial was a 40 ms, 120 dB white noise burst. In the pre-pulse (pp) + pulse trials, 20 ms white noise pre-pulse stimuli of 71 dB (pp6), 75 dB (pp10), and 83 dB (pp18) were presented for 100 ms before a 40 ms, 120 dB pulse. The non-stimulus (NS) trials presented the background noise only. The initial and the last blocks were both composed of six PA trials. Two middle blocks consisted of PA, pp + pulse, and NS trials. These trials were presented pseudo-randomly and were separated by 15-*sec* intertribal intervals on average (varying between 10 and 20 s). The percentage of PPI was evaluated using the following formula:% PPI = 100 × [(PA score) – (pp-P score)] / (PA score), where the PA score was the average of the PA value in the middle blocks.

The mice were randomly assigned into four groups: (1) saline control, (2) MK-801; (3) UPPTA (50 mg/kg) + MK-801, and (4) UPPTA (200 mg/kg) + MK-801. Each mouse in Groups 2–4 was received an acute administration of 0.3 mg/kg MK-801 dissolved in normal saline by i.p. injection 20 min prior to the PPI test. Each mouse in Groups 3 and 4 was received an acute oral administration of 50 and 200 mg/kg UPPTA dissolved in ddH_2O by p.o. 20 min prior to the MK-801 administration.

Results

Identification of Merck's TAs compositions and determination of their inhibitory activities against DAAO

We firstly tested the overall DAAO inhibitory potency of TAs supplied by Merck prior to the identification of their compositions. As shown in Fig. 2, Merck's TAs showed strong DAAO inhibition with an IC_{50} value of 0.601 μg/mL. We therefore isolated and structurally identified the composition of Merck's TAs. As shown in Table 1, the major components of the collected fractions of Merck's TAs were identified by HPLC-PDA-MS and NMR analyses and compared with reports in

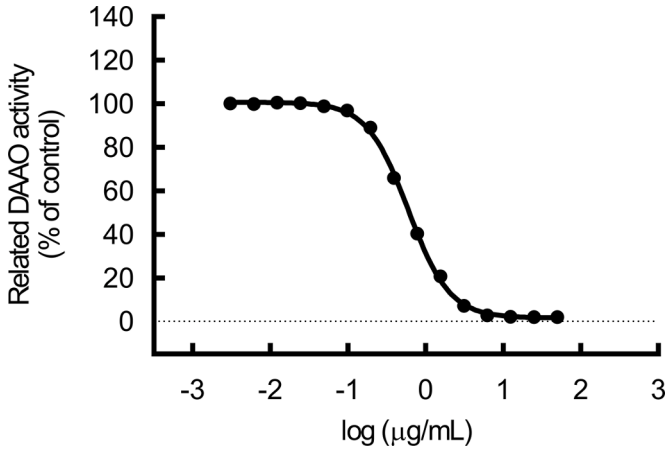

Fig. 2. Dose-response curve of Merck's TAs which is related to DAAO activity with an IC_{50} value of 0.601 μg/mL. Data were presented as means ± SD ($n = 3$ per concentration).

Table 1
Fractions with different numbers of galloyl moieties isolated from Merck's TAs and confirmed by HPLC-PDA-MS and NMR analyses.

Fraction	Molecular weight (grams/mol)	[M-H]- (*m/z*)	Degree of galloylation*	Compounds
5	170	169		Gallic acid (Caligiani et al., 2014)
9	484	483	2G*	1,6-di-*O*-Galloyl-β-D-glucopyranoside (Kashiwada et al., 1988)
13	636	635	3G*	1,2,6-Tri-*O*-galloyl-*β*-D-glucopyranoside (Nawwar et al., 1994)
15	788	787	4G*	1,2,3,6-Tetra-*O*-galloyl-*β*-D-glucopyranoside (Spencer et al., 1990)
18	940	939	5G*	1,2,3,4,6-Penta-*O*-galloyl-*β*-D-glucopyranoside (Cryan et al., 2013)
20	1092	1091	6G*	Mixture of 6 G tannic acids#
22	1244	1243	7G*	Mixture of 7 G tannic acids#
24	1396	1395	8G*	Mixture of 8 G tannic acids#
25	1548	1547	9G*	Mixture of 9 G tannic acids#
26	1700	1699	10G*	Mixture of 10 G tannic acids#

* G is an abbreviation for the galloyl group of the tannic acids. One G represents one galloyl moiety, 2 G represents 2 galloyl moieties, and so forth.
# Purified fractions of tannic acids with the same degree of galloylation.

the literature. For convenience, the galloyl group of TAs is abbreviated as G, i.e., 1 G represents 1 galloyl moiety. We subsequently evaluated the DAAO inhibitory potency of each Merck's TAs fraction. In Fig. 3, TAs with more than 6 G exhibited significantly stronger DAAO inhibitory activities at 300 nM. Moreover, as shown in Table 2, a positive correlation between galloyl moiety number and DAAO inhibiting potency was observed.

Comparisons of TA compositions and DAAO inhibitory activities from different commercial suppliers

The results of Merck's TAs showed promise in the inhibition of DAAO activities. We prepared our highly-purified UPPTA directly from *Rhus chinensis* using the procedures as described in Material and Methods. Subsequently, the compositions and DAAO inhibitory activities of the UPPTA and commercial TAs, of which the suppliers were Merck (*Quercus infectoria*) and Sigma (*Rhus chinensis*), were compared. The galloylation degree of TAs was identified based on their mass spectra together with literature data and quantified by the HPLC-PDA method. The HPLC chromatograms of TAs from the suppliers are illustrated in Fig. 4. The DAAO inhibitory activities of the commercial TAs and their compositions are provided in Table 3. As shown in Fig. 4 and Table 3, TAs extracted from *Rhus chinensis* contained much higher proportion of 5–12 G than from *Quercus infectoria,* resulting in stronger DAAO inhibitory potencies. Compared with the commercial TAs, the UPPTA contained the highest content of 5–12 G TAs with negligible content of impurities, contributing to the most potent DAAO inhibitory activities.

Table 2
The IC_{50} of DAAO inhibitory activities of TAs with different numbers of galloyl moieties.

Degree of galloylation	IC_{50} (μM)
3G*	8.474
4G*	1.576
5G*	0.496
6G*	0.375
7G*	0.256
8G*	0.337
9G*	0.240
10G*	0.215

* G is an abbreviation for number of the galloyl group of tannic acids.

The MTD study of TAs from different sources

To investigate the safety effect caused by the differences in composition, we assessed the oral toxicity of Sigma's TAs and the UPPTA. In the groups of Sigma's TAs, all mice received 1000 mg/kg Sigma's TAs displayed inactivity, sternal recumbency, altered respiration rate in 35 mins after dosing, and became moribund on day 2. The symptoms were not reversed during the study. All mice receiving 500 mg/kg or 750 mg/kg survived during the study whereas one-third mice displayed sternal recumbency in 35 mins after dosing. The MTD of Sigma's TAs was determined to be 750 mg/kg. On the other hand, in the groups of UPPTA, no test article-related toxic effects were noted in mice received 3000 mg/kg and 3500 mg/kg of the UPPTA. However, 3 out of 4 and 4 out of 4 mice were found dead in 4000 mg/kg and 4500 mg/kg groups,

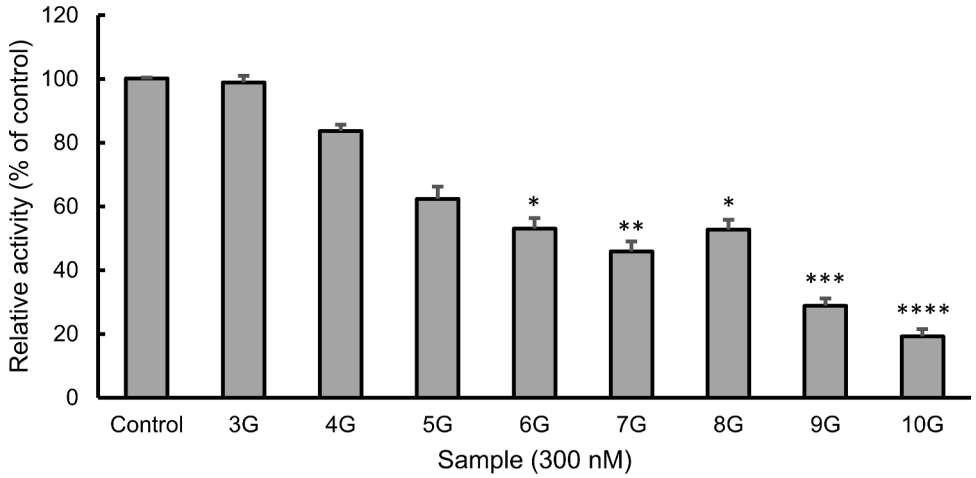

Fig. 3. Relative DAAO inhibitory activities of TAs with different numbers of galloyl moieties at 300 nM. G is an abbreviation for the galloyl group of TAs. Data were presented as mean ± S.E.M. ($n = 4$ per group) and analyzed with one-way ANOVA followed by Dunnett's multiple comparison test. The p values, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$, were compared to the control group.

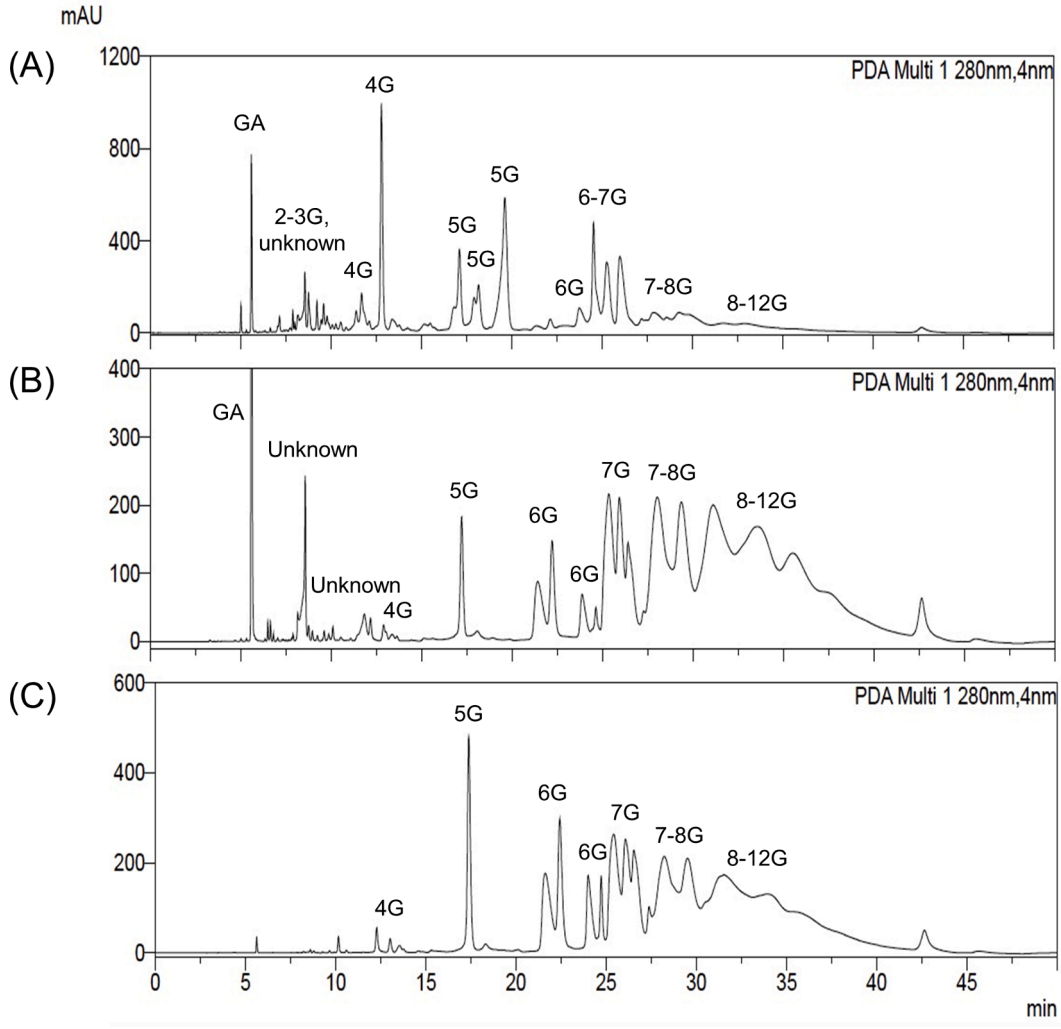

Fig. 4. The HPLC chromatograms of TAs from (A) Merck, (B) Sigma, and (C) UPPTA. The peaks of TAs with different degree of galloylation were noted on the diagram. GA: gallic acid; unknown: other impurity.

Table 3
Comparisons of tannic acid compositions and the inhibitory activities against DAAO from different suppliers.

Supplier	Merck	Sigma	SyneuRx
Source plant	*Quercus infectoria*	*Rhus chinensis*	*Rhus chinensis*
Grade	Pharmaceutical	ACS	Pharmaceutical
IC_{50} of DAAO (μg/mL)	0.601	0.424	0.333
Composition (%)			
Gallic acid (GA) (Impurity)	2.20%	5.25%	0.05%
Other unknown impurities	1.50%	3.05%	0.00%
1–4G*	21.18%	1.57%	1.71%
5–7G*	48.57%	20.94%	36.73%
8–12G*	26.55%	69.19%	61.51%

* G is an abbreviation for number of the galloyl group of tannic acids.

respectively. Based on these results, the MTD of UPPTA was considered to be 3500 mg/kg. The much higher MTD of UPPTA compared with that of Sigma's TAs could be attributable to the very low impurity content in UPPTA.

The effects of TAs from different sources on locomotion in MK-801 treated mice

MK-801, an antagonist of the NMDA receptor, has been used in many NMDA hypofunction-induced symptoms of CNS diseases, including stereotypic behaviors, working memory impairment, and sensorimotor function abnormalities (Lee and Zhou, 2019). The effects of different sources of TAs in MK-801-insulted mice model were evaluated by an open field test, a Barnes maze test, and a pre-pulse inhibition test. In the open field test, the UPPTA treated mice displayed rescue effects on MK-801-inhuced hyper-locomotion ($p < 0.05$), whereas the Merck's TAs group did not, as shown in Fig. 5.

The effects of TAs from different sources on MK-801-disrupted working memory in mice

We subsequently evaluated the rescue effects of different sources of TAs on MK-801-disrupted working memory by Barnes maze test. The MK-801 treated mice displayed impaired working memory compared with control ($p < 0.01$). The UPPTA group, but not the Merck's TAs group, displayed significantly improved memory retrieval in the MK-801 treated mice in the Barnes maze task ($p < 0.05$), as shown in Fig. 6.

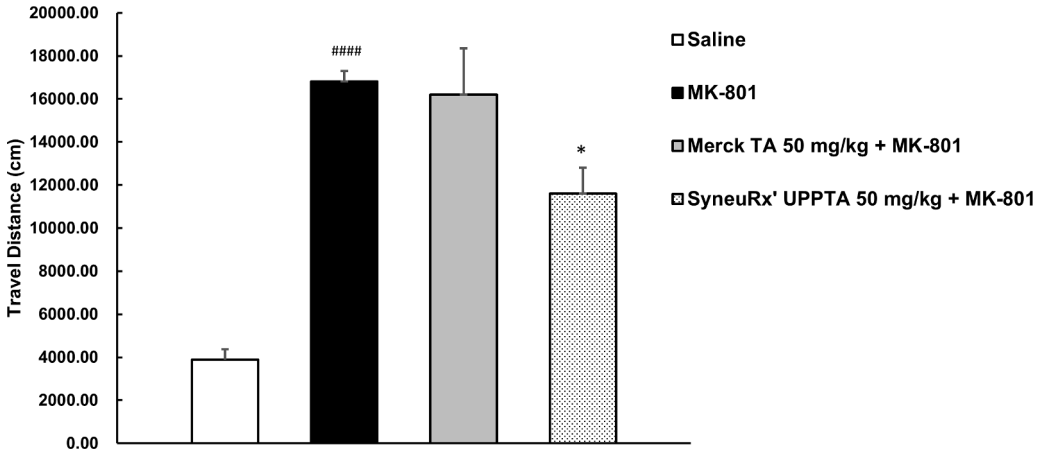

Fig. 5. Effects of Merck TAs and UPPTA on reducing MK-801 induced hyper-locomotion. Data was presented as mean ± S.E.M. ($n = 5$–8 per group) and analyzed with one-way ANOVA followed by Dunnett's multiple comparison test. The p value, $^{\#\#\#\#}p < 0.0001$ was compared to the saline group. The p value, $^{*}p < 0.05$ was compared to the MK-801 group.

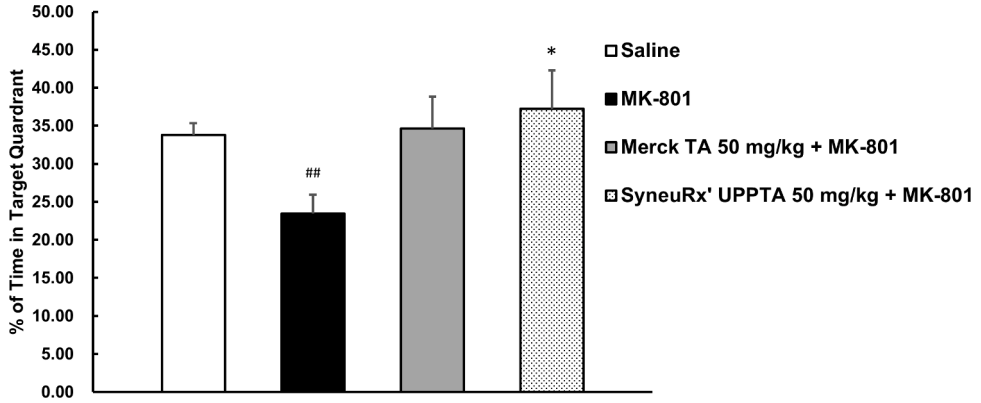

Fig. 6. Effects of Merck TAs and UPPTA on improving MK-801 disrupted working memory in Barnes maze test. Data was presented as mean ± S.E.M. ($n = 5$–8 per group) and analyzed with Student's unpaired *t*-test. The p value, $^{\#\#}p < 0.01$ was compared to the saline group. The p value, $^{*}p < 0.05$ was compared to the MK-801 group.

The effects of UPPTA on pre-pulse inhibition in MK-801-treated mice

Due to its higher bioactivity compared with the commercial TAs, the UPPTA was chosen to be tested in the PPI test. As illustrated in Fig. 7, MK-801 (0.3 mg/kg) treated mice showed robust PPI deficits in all pre-pulse intensities ($p < 0.001$). Both 50 mg/kg and 200 mg/kg UPPTA significantly improved the MK-801-induced PPI deficits under 75 db and 83 db intensities. A ceiling effect at a dose of 50 mg/kg was observed

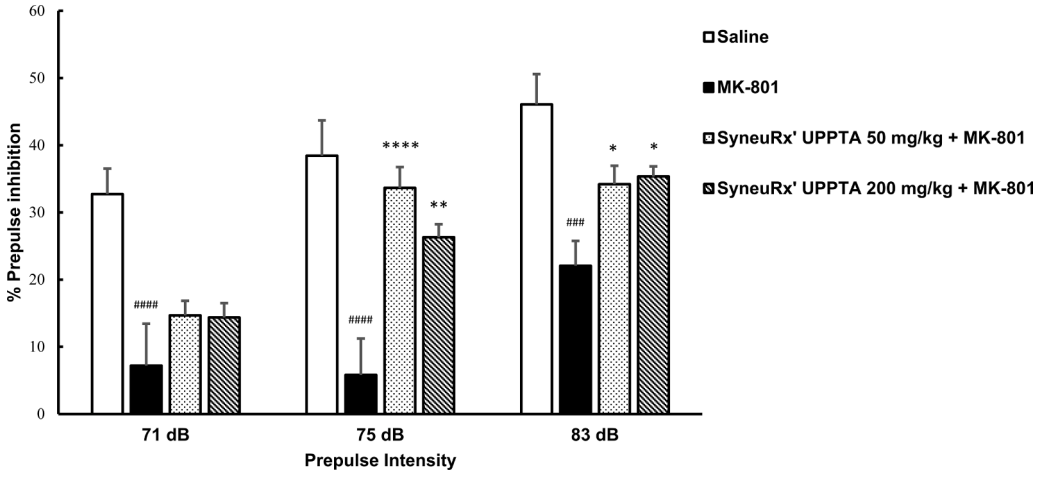

Fig. 7. Effects of UPPTA on ameliorating the MK-801-induced pre-pulse inhibition deficit. Data was presented as mean ± S.E.M. ($n = 4$–5 per group) and analyzed with two-way ANOVA followed by Dunnett's multiple comparison test. The p values, $^{\#\#\#}p < 0.001$ and $^{\#\#\#\#}p < 0.0001$, were compared to the saline group. The p values, $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{****}p < 0.0001$, were compared to the MK-801 group.

since there was no significant difference between tested doses.

Discussion and conclusion

In this study, we evaluated DAAO inhibitory activities of TAs with different compositions in nature which are from different sources. The TAs extracted from *Rhus chinensis* were found to contain a much higher percentage of 5–12 G and demonstrated a much stronger DAAO inhibition potency than from *Quercus infectoria*. In particular, the UPPTA contained the highest composition of 5–12 G of TAs than the other two sources. Moreover, the studies conducted in MK-801-induced NMDAR hypofunction mice model revealed that TAs, especially the UPPTA, attenuated MK-801-induced hyper-locomotion, PPI deficit, and memory impairment through inhibiting DAAO. These results further confirmed the superiority of UPPTA, endorsing it as a novel and strong candidate for further development for the therapy of CNS disorders.

The research of TAs in CNS disorders has been mainly focused on Alzheimer's disease. Mori et al. described that TAs, as β-secretase inhibitors, mitigated Alzheimer-like cerebral β-amyloid deposits by attenuating β-secretase activity and neuroinflammation (Mori et al., 2012). TAs also inhibited in vitro aggregation of tau peptide, a critical component causing intracellular neurofibrillary tangles (Yao et al., 2013). Intriguingly, the inhibitory activity of TAs against DAAO and the clicnial potential of TAs for psychotic symptoms, in addition to Alzheimer's disease, have been evidenced in our study. Our findings have indicated that TAs are potential therapeutic agents for treating schizophrenia and the behavioral and psychological symptoms of dementia, since our previous clinical studies found the positive correlation between D-serine levels in synapses and NMDAR functions (Lane et al., 2013; Lin et al., 2014).

Despite such well-known properties of TAs, no study further purified naturally sourced crude TAs products until now. As a result of our manufacturing process, the UPPTA has scarce impurity content and shows high safety profile while the commercial TAs have substantial impurities (Table 3). The MTD of UPPTA was determined to be 3500 mg/kg in mice, indicating its high safety margin. A similar result was observed in our previous 14-day repeated dose toxicology studies, the MTD and non-observable-adverse-effect level (NOAEL) of UPPTA were determined to be 4000 mg/kg and 2000 mg/kg in rats (Shih et al., 2022).

Although Picchioni's study (1966) noted that the TAs in barium enema showed potential hepatic toxicity (Picchioni et al., 1966), we concluded that the toxicity is likely caused by the botanic source and the compositions of TAs. Unlike the UPPTA originated from Chinese gallnut, the TAs in barium enema were isolated from the nutgalls of Aleppo oak (*Quercus*). The median LD_{50} in rats of Chinese tannin is approximately doubled to the number of Aleppo tannin (2800 mg/kg vs. 1550 mg/kg) (FAO Nutrition Meetings Report Series, 1970). Our analysis also reveals that the UPPTA is composed of 5–12 G TAs, but Aleppo tannin only contains 2–6 G TAs (Zajacz et al., 2007). Besides, no clinical or preclinical evidence is reported regarding the hepatic toxicity of Chinese tannin (Chang, 1997; Jiang and Chi, 1996; Sang, 1994). In addition to the source, the TAs in barium enema were not purified and identified, while UPPTA was purified under modern purification process with rigorous monitoring. These differences may lead to toxicity shown in Picchioni's study but not in the UPPTA.

Overall, the UPPTA is a promising therapeutic candidate originating from an enriched history of human medical use. Removing the impurities and low galloylation TAs through purification, the UPPTA has shown excellent safety profile and superior inhibition against DAAO. In the in vivo studies, the UPPTA has been able to ablate MK-801-induced hyper-locomotion, PPI deficit, and memory impairment. The UPPTA can be developed for NMDA-impaired CNS disorders therapy.

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CRediT authorship contribution statement

Yi-Wen Mao: Conceptualization, Project administration, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Lu-Ping Lu:** Methodology, Formal analysis, Investigation, Data curation. **Po-Chang Shih:** Writing – original draft, Writing – review & editing. **Jui-Fu Hsiao:** Writing – review & editing. **Vivian Wu:** Conceptualization, Project administration, Methodology, Formal analysis, Investigation, Data curation. **Guochuan E. Tsai:** Conceptualization, Project administration, Methodology, Formal analysis, Investigation, Data curation, Supervision, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors are the employees of SyneuRxInternational (Taiwan) Corp. when the manuscript was drafted. Patent portfolio of UPPTA was assigned to SyneuRx International (Taiwan) Corp. The trademark is Pentarlandir®and Airnecflu®.

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