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ORIGINAL PAPER

Gastrodin Ameliorates Memory Deficits in 3,3′-Iminodipropionitrile-Induced Rats: Possible Involvement of Dopaminergic System

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Abstract 3,3′-Iminodipropionitrile (IDPN), one of the nitrile derivatives, can induce neurotoxicity, and therefore cause motor dysfunction and cognitive deficits. Gastrodin is a main bioactive constituent of a Chinese herbal medicine (*Gastrodia elata Blume*) widely used for treating various neurological disorders and showed greatly improved mental function. This study was designed to determine whether administration of gastrodin attenuates IDPN-induced working memory deficits in Y-maze task, and to explore the underlying mechanisms. Results showed that exposure to IDPN (150 mg/kg/day, v.o.) significantly impaired working memory and that long-term gastrodin (200 mg/kg/day, v.o.) could effectively rescue these IDPN-induced memory impairments as indicated by increased spontaneous alternation in the Y-maze test. Additionally, gastrodin treatment prevented IDPN-induced reductions of dopamine (DA) and its metabolites, as well as elevation of dopamine turnover ratio (DOPAC + HVA)/DA. Gastrodin treatment also prevented alterations in dopamine D2 receptor and dopamine transporter protein levels in the rat hippocampus. Our results suggest that long-term gastrodin treatment may have potential therapeutic values for IDPN-induced cognitive impairments, which was mediated, in part, by normalizing the dopaminergic system.

Keywords Gastrodin · 3,3′-Iminodipropionitrile · Working memory impairments · Dopamine · Dopamine D2 receptor · Dopamine transporter

Abbreviations

IDPN	3,3′-Iminodipropionitrile
GAS	Gastrodin
GE	*Gastrodia elata* Blume
DA	Dopamine
DAT	Dopamine transporter
DOPAC	3,4-Dihydroxyphenylacetic acid
HVA	Homovanilic acid
HPLC	High-performance liquid chromatography

Introduction

3,3′-Iminodipropionitrile (IDPN) is one of nitriles, which are extensively used for manufacture of fibers, plastics, dyes, resins and pharmaceuticals, and may lead to neurobehavioral

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abnormalities in humans and experimental animals [1]. IDPN-induced behavioral syndrome encompasses repetitive head movements, retropulsion, circling and hyperactivity [2]. Accumulating evidence suggests that IDPN causes damages to the central nervous system, particularly in midbrain [3], olfactory bulbs [4], cingulate cortex and hippocampus [5]. Previous research also showed that learning and memory deficits in rodents treated with IDPN, further supporting a role of higher levels of central nervous system in IDPN neurotoxicity [6]. In addition, IDPN could disrupt performance of olfactory discrimination learning and produce cognitive deficits in T-maze learning in infant rats [7]. Moreover, IDPN-induced dyskinesia has demonstrated markedly alterations in the serotonin, dopamine (DA) and acetylcholine neuronal systems [8]. However, the underlying mechanisms for IDPN-induced cognitive impairments are not well understood.

Gastrodia elata Blume (GE) is one of the most popular traditional medicinal herbs in South-east Asian countries. Gastrodin is a main bioactive component of GE, and has been widely used clinically for treatments of neurasthenia, dizziness, epilepsy, migraine and dementia [9]. Gastrodin could penetrate the blood–brain barrier and enter the central nervous system [10]. A considerable amount of evidence has shown that gastrodin could exert neuroprotective pharmacological effects [11–13]. Gastrodin could protect not only primary cultured rat hippocampal neurons against amyloid-beta peptide-induced neurotoxicity [14], but also apoptotic dopaminergic neurons in a toxin-induced Parkinson's disease model [15]. Furthermore, there is increasing evidence that gastrodin would improve learning and facilitate memory [16, 17]. For instance, gastrodin can attenuate learning deficits induced by forced-swimming stress in the inhibitory avoidance task and Morris water maze [18] and rescue impairments of synaptic plasticity induced by lead in the rat hippocampus [19]. Based on these findings, we hypothesized that gastrodin exerts protective effects on working memory impairments caused by IDPN in rat model.

Brain dopamine has long been implicated in cognitive control processes [20, 21]. Experimental and clinical studies based on dopaminergic pathology, depletion or medication indicate that DA is involved in working memory [22]. Hippocampal dopaminergic innervation arises mainly from the midbrain, and is part of the mesolimbic dopaminergic pathway [23, 24]. Importantly, it is manifested that DA innervations of the hippocampus are involved in spatial working memory [25–27]. Additionally, dopamine transporter (DAT) is a protein located at nerve terminals, and plays a pivotal role in neuronal homeostasis regulating extracellular neurotransmitter concentrations [28]. A previous study revealed that preweaning Manganese exposure led to spatial learning and memory deficits associated with decreased DAT in nucleus accumbens and dorsal striatum, while eight-arm radial maze training increased the level of D2 protein in the prefrontal cortex [29]. Of particular relevance for the current study, D2 receptor belongs to the D2-like (D2, D3 and D4 type) subfamily of dopamine receptors, and is expressed in the hippocampal dentate gyrus [30, 31]. A growing body of evidence from human [32–34] and animal [26, 35–37] studies suggests that dopamine D2 receptor may be involved in working memory. Recent investigations have shown that chronic haloperidol-induced spatial memory deficits were correlated with up-regulation of D2 receptor in the caudate putamen of mouse [37], and that over activation of D2 receptor during adolescence impaired working memory and neural circuits [38]. Despite these observations, it remains presently unknown to the direct involvement of DA, D2 receptor and DAT in improvement of working memory impairments with gastrodin treatment in the hippocampus.

Based on the aforementioned studies, we first examine whether prolonged gastrodin treatment is capable of rescuing IDPN-induced cognitive deficits in short-time working memory. Additionally, we investigate alternations in DA levels, dopamine turnover ratio and D2 receptor; correlated changes in DAT protein expression is further determined by high-performance liquid chromatography with electrochemical detection (HPLC-ECD) and western blot analysis in IDPN-induced rats.

Materials and Methods

Animals

Wistar male rats of 4-week-old (weighing 100–120 g) were obtained from the Laboratory Animal Center of Shandong University. Rats were housed in standard plastic cages (two animals in per cage) under controlled temperature (22 ± 1 °C) and humidity (50 ± 5 %) conditions with a 12-h light/dark cycle (lights on from 0600 to 1800 hour). Food and water were available ad libitum throughout the experiments. All procedures were approved by the Shandong University Animal Care and Use Committee and were carried out in compliance with the National Institutes of Health guide for the care and use of Laboratory animals (Publication No. 85-23, revised 1985). All efforts were made to reduce the number of rats used and their suffering.

Drug Administration and Experimental Groups

IDPN, Dopamine HCl (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Gastrodin was obtained by Kunming Pharmaceutical Co. (Yunnan, China).

Rats were randomly assigned to one of four conditions (8 rats per group): (1) control group received saline treatment at a dose of 10 ml/kg/day during the experimental period (normal saline); (2) saline plus gastrodin group (control plus GAS); (3) IDPN alone-treated group and (4) IDPN-treated received gastrodin group (IDPN plus GAS). The IDPN-treated group was received intragastric administration with IDPN at a dose of 150 mg/kg/day (dissolved in normal saline) for 7 consecutive days to establish IDPN-induced neuropathy. On the eighth day, rats from two groups (saline plus GAS and IDPN plus GAS) were only administrated with gastrodin in a dosage of 200 mg/kg/day (dissolved in normal saline) for 8 consecutive weeks. We chose a 200 mg/kg injection regimen as based upon previous results [39]. All treatments consisted of an intragastrical injection between 9:00 a.m. and 11:00 a.m.

Rats were submitted to Y-maze task 60 min after the last drug administration. Rats were then sacrificed following the behavioral test. Brains were immediately removed, and then the hippocampi were dissected according to the rat atlas and frozen at −70 °C for neurochemical and Western blot analyses.

Y-Maze Task

Spontaneous alternation behavior, which reflected a short term working memory, was assessed in a Y-maze based on the methods of Sarter et al. [40]. The maze used in the present study consisted of black plastic with three arms (50 cm long, 20 cm high and 10 cm wide) and an equilateral triangular central area. The walls of each arm had geometric shapes that provided spatial cues. Each rat was placed in the distal end of one arm, and allowed to explore the apparatus for 8 min. 'An arm entry' was counted when all four paws of the rat were within one arm. The number of arm entries was used as an indicator of locomotor activity. Spontaneous alternation behavior was defined as multiple entries into the three different arms on overlapping triplet sets, i.e., ABC, BCA, or CAB but not CAC. The number of maximum spontaneous alternation behaviors was the total number of arms entered minus two and the percentage of spontaneous alternation was calculated as (actual alternations/maximum alternations) × 100.

Quantification of Dopamine and Dopamine Turnover

The concentrations of DA, DOPAC and HVA from the hippocampus were assayed using HPLC with electrochemical detection. Following sample collections, 300–800 μl of 0.1 M perchloric acid was added to the tissues and the mixture was homogenized for 1 min at 4 °C before neurochemical evaluation was performed. The homogenates were centrifuged for 10 min at 15,000×*g* and 10 μl of the supernatant was injected into the chromatograph. The measurements were performed by HPLC system coupled to 2465 Pulsed ECD (Waters, Meliford, USA). The analyzed compounds were separated on a reversed-phase C18 column (2.1 mm × 50 mm, 1.9 μm particle size) (Thermo, Rockford, IL, USA). The mobile phase consisted of 0.15 M citric acid-sodium citrate buffer solutions (pH 3.5), 1 mM sodium 1-heptanesulfonate, 0.1 M EDTA·2Na and 15 % (v/v) methanol. Elution was carried out at a flow rate of 0.1 ml/min and the working electrode potential was set at 0.8 V. The column temperature was 28 °C. The concentrations of the dopamine and its metabolites in the samples were calculated and expressed as ng/mg protein. The turnover of dopamine (DOPAC + HVA/DA) was also reported.

Western Blot Analysis

Hippocampus samples were homogenized in 100 μl of homogenizing buffer containing 50 mM Tris (pH 7.4),150 mM NaCl, 1 % Triton X-100, 1 % sodium deoxycholate, 0.1 % SDS, sodium orthovanadate, sodium fluoride, EDTA, leupeptin and 1 mM PMSF. Insoluble material was removed by a 10 min centrifugation at 10,000*g* at 4 °C. Protein concentration in supernatants was determined using BCA protein assay kit (Cowin Biotech, Beijing, China). A quantity of 30–50 μg total proteins was loaded onto a 10 % SDS-PAGE gel. After electrophoresis, the separated proteins were transferred onto the PVDF membrane (Immobilon-P, Millipore, Bedford, MA, USA) and blocked in blocking buffer PBST (5 % nonfat dried milk in Phosphate Buffered Saline containing 0.1 % Tween-20) for 1 h at room temperature. Subsequently, the membranes were probed with the primary antibodies overnight at 4 ° C: anti-Dopamine D2 receptor (1:1,000) (Millipore, Chemicon, USA), anti-Dopamine transporter (1:1,000) (Abcam, Cambridge, UK) and anti-β-actin (1:1,000) (ZSGB-Bio, Beijing, China). Secondary antibodies were horseradish peroxidase conjugated to goat anti-rabbit/mouse IgG (1:10,000) (ZSGB-Bio, Beijing, China). Bound antibodies were visualized with enhanced chemiluminescence detection system (Pierce, Rockford, IL) and analyzed semiquantitatively using the National Institutes of Health ImageJ program.

Statistical Analysis

Data were analyzed and graphs were created using Prism 5 (GraphPad Software, La Jolla, CA). Values are presented as mean ± SEM. All data were analyzed statistically by one-way analysis of variance (ANOVA) for multiple comparisons followed by Tukey's post hoc test. Pearson's correlation coefficient and regression analysis were used in order to evaluate the connection between spontaneous

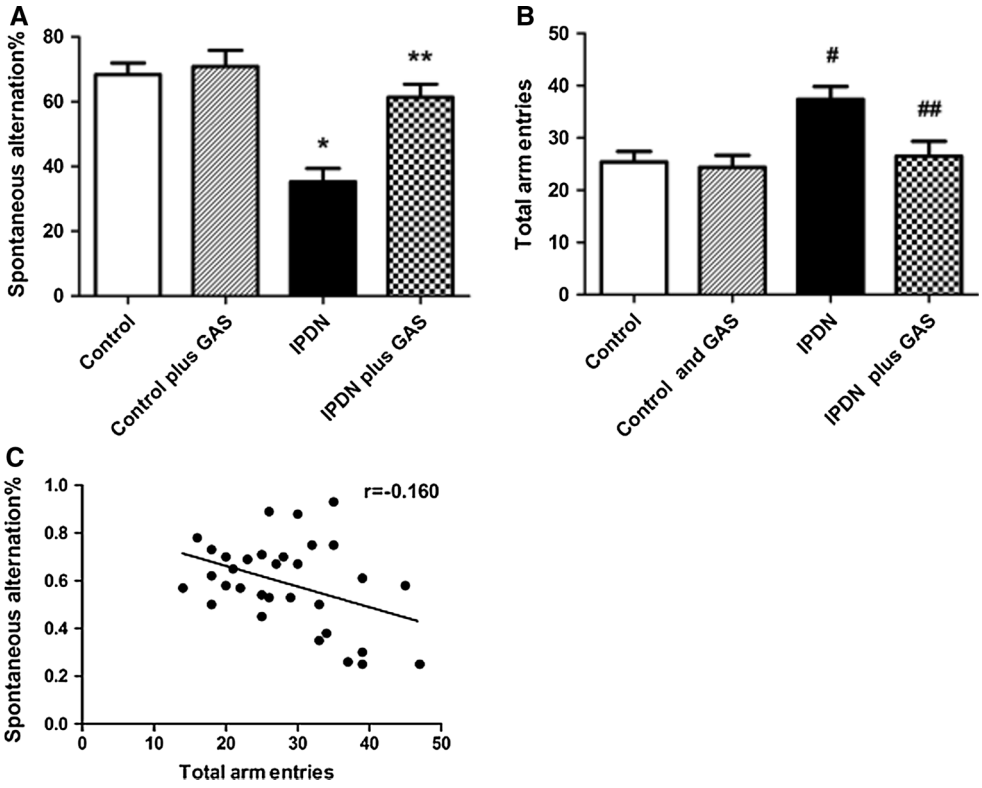

Fig. 1 Effects of administration of gastrodin on IDPN-induced memory impairment of spontaneous alternation behavior (**a**) and number of arm entries (**b**) in the Y-maze test. Data represent the mean ± SEM. (n = 8 per group). $^{*}p < 0.001$, $^{\#}p < 0.01$ versus the control group. $^{**}p < 0.001$, $^{\#\#}p < 0.05$ versus the IDPN-treated group (one-way ANOVA followed by Tukey's post hoc test). **c** A significant negative correlation (linear regression, Pearson's r) between spontaneous alternation percentage and total arm entries

alternation percentage and total arm entries, DA as well as its metabolites contents. $p < 0.05$ was considered indicative of statistical significance.

Results

Effect of Gastrodin on Working Memory in the Y-Maze Task

The effects of gastrodin on spontaneous alternation behaviors (alternation rate and total arm entries) in the Y-maze are shown in Fig. 1a, b. Post-hoc analysis revealed that administrations of IDPN produced a significant decrease in spontaneous alternation percentage ($p < 0.001$, Fig. 1a) as compared to the control rats. However, we observed a significant increase of spatial memory in gastrodin-treated group exposed to IDPN ($p < 0.001$, Fig. 1a), indicated by an increase of spontaneous alternation percentage compared to IDPN-induced group.

We also calculated the number of arm entries as an indicator of locomotor activity. As shown in Fig. 1b, rats subjected to IDPN showed increased total arm entries when compared with control rats ($p < 0.01$), whereas these increases were reversed by long-term gastrodin treatment ($p < 0.05$).

Besides, we obtained a significant negative correlation in rats: spontaneous alternation percentage versus total arm entries (n = 24, r = −0.160, $p < 0.05$, Fig. 1c).

Quantification of DA and DA Turnover

The results obtained in neurochemical data are shown in Fig. 2a, b. In the hippocampus IDPN induced significant the decreases in DA ($p < 0.001$) and its metabolites DOPAC ($p < 0.001$) and HVA ($p < 0.001$) levels (Fig. 2a), while (DOPAC + HVA)/DA ratio was higher compared with those in the control group ($p < 0.05$) (Fig. 2b). These decreases were attenuated by treatment of gastrodinin DA ($p < 0.001$), DOPAC ($p < 0.01$), and HVA ($p < 0.001$) levels (Fig. 2a). Administration of gastrodin significantly reduced (DOPAC + HVA)/DA ratio ($p < 0.05$) (Fig. 2b) as compared with IDPN-induced group.

Importantly, significant positive correlations were evidenced by determination of the linear regression between spontaneous alternation percentage versus DA contents (n = 24, r = 0.430, $p < 0.001$) (Fig. 3a) and spontaneous alternation percentage versus HVA contents (n = 24, r = 0.481, $p < 0.001$) (Fig. 3b) in the hippocampus.

Effect of Gastrodin on D2 Receptor and DAT Expression

The content of dopamine D2 receptor within hippocampus regions was measured following administration of gastrodin as a means to assess the possible involvement of cognitive-enhancing factors in the action of gastrodin. Post-hoc analysis confirmed that the D2 receptor protein levels in the

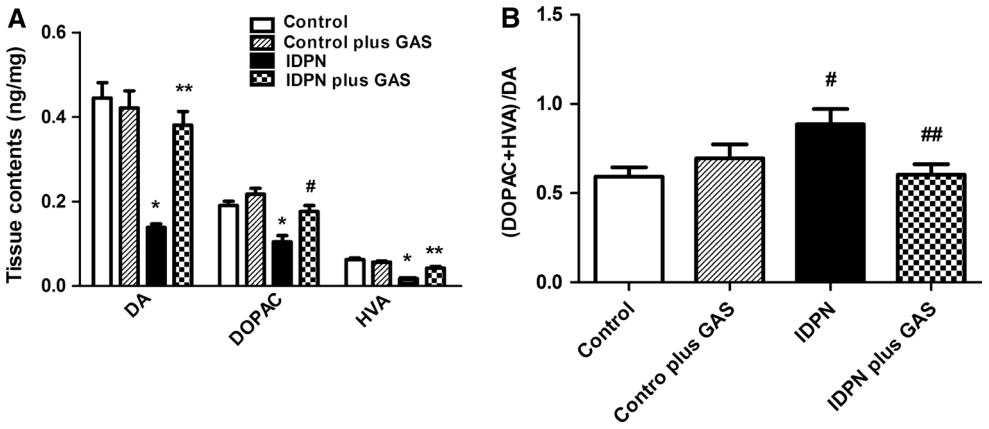

Fig. 2 Effects of gastrodin on IDPN- induced changes in DA and its metabolites (ng/mg protein) in the hippocampus (**a**). Values are represented as Mean ± SEM; n = 8, One way ANOVA followed by post hoc Tukey's test analysis.*$p < 0.001$ represents when compared to normal group; **$p < 0.001$, $^{\#}p < 0.01$ represents as compared to the IDPN-treated group. Effects of gastrodin treatment on turnover of DA in the hippocampus of the IDPN-treated rats (**b**). Data are represented the mean ± SEM, n = 8. $^{\#}p < 0.05$ versus control group; $^{\#\#}p < 0.05$ versus IDPN-treated group (one-way ANOVA followed by Tukey's post hoc test)

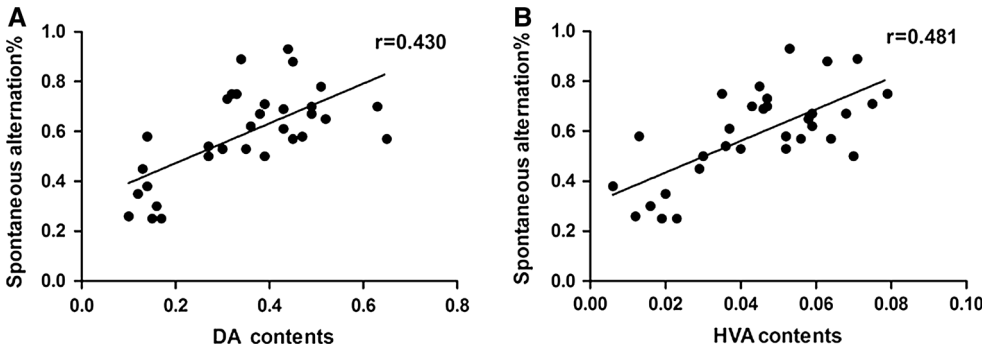

Fig. 3 Correlation between spontaneous alternation percentage versus DA contents (**a**), spontaneous alternation percentage versus HVA contents (**b**) in all rats. The best fit slopes correspond to the Pearson correlations

hippocampus of IDPN-induced rats were significantly increased compared with saline-treated rats ($p < 0.001$, Fig. 4a). Whereas long-term administration of gastrodin significantly reversed the increase of D2 receptor protein levels in the hippocampus as compared to IDPN-treated group ($p < 0.05$, Fig. 4a).

We next investigated whether DAT in the hippocampus is involved in the memory-facilitating effects induced by gastrodin. The results showed that IDPN exposure significantly decreased the DAT protein levels ($p < 0.001$, Fig. 4b) compared with the corresponding controls. However, long-term treatment with gastrodin effectively prevented IDPN-induced the decrease of DAT protein levels in the hippocampus ($p < 0.05$, Fig. 4b).

Discussion

Our study demonstrated that long-term administration of gastrodin could significantly mitigate the deleterious effects of IDPN on the short-time working memory, as tested by Y-maze tasks. Gastrodin treatment prevented IDPN-induced reduced levels of DA and its metabolites, as well as elevated dopamine turnover rate (DOPAC + HVA)/DA. In addition, gastrodin treatment also prevented alterations in D2 receptor and DAT protein levels in the hippocampus of IDPN-treated rats.

In this study, IDPN-induced memory deficits were tested by Y-maze test, a specific and sensitive test for spatial recognition memory, in rats administered gastrodin for 2 months. Consistent with a previous study [41], our data showed that rats exposed to IDPN demonstrated decreased spontaneous alternation in the Y-maze, as compared to saline-treated control group, suggesting that administration of IDPN produced markedly short-term working memory deficits in rats. Additionally, the present results confirmed and extended prior findings that there was a negative correlation between arm entries and spontaneous alternation behavior [40]. Consistently, our data showed that the numbers of arm visited were increased while spontaneous alternations were decreased, which was confirmed by IDPN-induced hyperactivity of rats. It is of high importance that gastrodin treatment could reversed these deficits as shown by increased spontaneous alternation in IDPN-

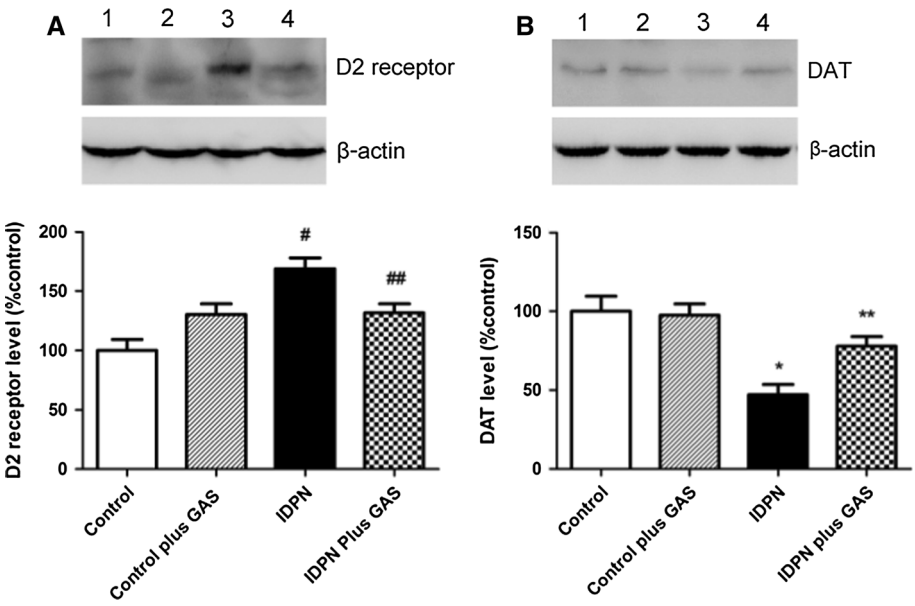

Fig. 4 Effect of gastrodin on the expressions of D2 receptor and DAT. Western blot analysis of D2 receptor (**a**) and DAT (**b**) expression in the hippocampus. *Band 1* control group; *Band 2* control plus GAS; *Band 3* IDPN; *Band 4* IDPN plus GAS. Normalized intensity bands of D2 receptor and DAT are presented as the mean ± SEM of at least three separate experiments. Results showed the percentage changes from the saline treated control group. $^{\#}p < 0.001$, $*p < 0.001$ compared with the saline-treated group; $^{\#\#}p < 0.05$, $**p < 0.05$ compared with the IDPN-treated group (one-way ANOVA followed by Tukey's post hoc test)

exposed rats, indicative of a protective function of gastrodin in working memory deficits induced by IDPN. The data were also in accordance with previous studies showing gastrodin and HBA, which was several active ingredients of GE, can facilitate memory consolidation and retrieval, but not acquisition [16]. The aqueous extract of GE improved D-galaxies induced memory impairments in mice and performance on a passive-avoidance task in senescent mice [42]. Taken together, the present study demonstrated, for the first time, ameliorative effects of long-term gastrodin treatment on IDPN-induced working memory impairments. However, further researches are required to more specifically target whether acute gastrodin treatment is involved in facilitating memory effects in IDPN-induced rats in the Y-maze task.

In our study, long-term gastrodin treatment prevented IDPN-induced reductions in the DA and its metabolites, as well as elevation of the DA turnover ratio (DOPAC + HVA)/DA. Similar results have been observed by study conducted by Kawada et al. [43], in which intraperitoneal injection of IDPN to male SD rats has led to decreased levels of DA and its metabolites. The dopaminergic system of IDPN-treated rats showed hypofunction in the hippocampus. Indeed, our data validated prior observations that prolonged GE treatment significantly increased DA concentration and decreased DA turnover in the striatum in the rats forced-swimming test [44] and in methamphetamine-treated rats [45]. Furthermore, several studies have shown that disturbances in dopaminergic systems induce learning and memory impairment in rats and in human imaging studies. For instance, it was previously reported that hippocampal injection of 6-hydroxydopamine caused selective lesions of the mesencephalic dopaminergic system, which induces learning and memory impairments [46]. Mehta et al. [47] also revealed that reduced synthesis and release of DA by tyrosine and phenylalanine depletion would trigger impairments in spatial working memory, which measured by [^{11}C]raclopride positron emission tomography. Importantly, Pioli et al. [48] demonstrated that lesions and other manipulations of mesocortical dopamine pathways could change performance in spontaneous alternation paradigms. In agreement with these findings, we obtained significant positive correlations between spontaneous alternation percentage versus DA and HVA contents from the hippocampus of rats. These data suggest that the increase of spontaneous alternation percentage in IDPN-treated rats could be correlated with the involvement of gastrodin in memory-facilitating effects. Of note, we also found that administration of gastrodin significantly improved memory deficits caused by IDPN. These results raise the possibility that prolonged gastrodin treatment reverses working memory dysfunction induced by IDPN was in part, due to rebounded DA and HVA levels in the hippocampus, which attributed to the protective effects of long-term gastrodin treatment.

In a complementary fashion, one potential mechanism that might explain memory-enhancing effects of gastrodin has been reported by Manavalan et al. [13], where they have advanced the hypothesis that tianma could promote neuroregenerative processes by inhibiting stress related proteins and mobilizing neuroprotective genes with various regenerative modalities and capacities related to neuro-synaptic plasticity. Another observation has revealed that GE attenuated methamphetamine-induced dopaminergic toxicity via inhibiting oxidative burdens [45]. Therefore, it is possible that long-term gastrodin treatment attenuates IDPN-induced neurotoxicity via one or more of these mechanisms, which requires further studies both in vivo and in vitro.

A significant evidence suggests that D2 receptor is involved in the dyskinetic movement of IDPN-treated rats [49]. Previously published data revealed that the density of D2 receptors in the corpus striatum did not differ between the IDPN-treated rats and the control ones using [^{3}H]-spiperone as a radioligand [8]. However, our results indicated that the levels of D2 receptor expression in IDPN-induced group were increased significantly, when compared to the control group. Possible explanations for the discrepant findings include differences in the dosing regimen, research methodologies, or involvements of different brain areas. Further studies using quantitative receptor autoradiography and immunohistochemical staining may help to test those differences. Importantly, rats received long-term gastrodin treatment showed unaffected D2 receptor expression, which was elevated by IDPN. In addition, D2 receptors modulate synaptic transmission, and are important for various brain functions, including learning and working memories [38]. As previously mentioned [26], injections of a D2 receptor antagonist raclopride into the rat hippocampus was found to impair learning, whereas a D2 agonist quinpirole attenuated this impairment, suggesting the role of hippocampal D2 receptor activity on memory performance. Another investigation indicated that spatial working memory accuracy was reduced by D2-like antagonist raclopride in Rhesus Monkeys [35]. Recently, in an elegant study conducted by Jia et al. [38], it was observed that adolescent D2 receptor hyperactivity impaired working memory in spontaneous Y maze test. In light of these findings, it is not surprising that our results indicated that the facilitating effects of long-term gastrodin treatment on working memory were accompanied by alterations in D2 receptor levels in the hippocampus of rats.

The dopamine transporter (DAT) is a high affinity transmembrane protein responsible for dopamine reuptake from the synaptic cleft and termination of dopaminergic transmission, which has thought to be implicated in cognitive function [50]. Specifically, Volkow et al. [51] previously pointed out that reduction of DAT in the methamphetamine abusers was associated with poor memory performance. It was demonstrated that spontaneous alternation in Y-maze was significantly decreased in DAT knockout mice [52], which reflected as hyperlocomotion in behavior [53]. In particular, emerging evidence indicated that mice expressing markedly reduced striatal DAT exhibit increased locomotor activity [54]. In addition, Wang et al. revealed that levels of DAT mRNA and protein expression were decreased in the striatum in IDPN-induced rats [55]. In accordance with these previous views, we found that IDPN decreased DAT levels in the hippocampus, rats suffering IDPN neurotoxicity, consequently, displayed impairments in working memory and exhibited increased locomotor activity. However, long-term gastrodin treatment prevented the attenuation of DAT levels and relative behavioral deficiency that was produced by IDPN. Thus, our results indicated that long-term gastrodin treatment could reverse working memory impairments induced by exposure to IDPN.

Gastrodin treatment was capable of reversing alterations of D2 receptor, DAT levels, and ameliorated the reduction of DA levels induced by IDPN. While the mechanism of alternations in D2 receptor and DAT levels is presently unknown, it is possible that the down-regulation of DAT protein may act as a long-term compensatory mechanism for maintaining DA input [56], or that dysregulation of DAT via dopamine D2 autoreceptors triggers anomalous dopamine efflux [57].Therefore, on the basis of our results and several lines of other evidences, further investigation is required to elucidate the molecular mechanisms underlying the DAT and potsynaptic/presynaptic D2 receptor interactions in IDPN-induced rats.

In summary, the present study has demonstrated that long-term gastrodin treatment significantly improves working memory deficits induced by IDPN administration in the hippocampus of rats, and these beneficial effects are, at least partially, mediated through ameliorating reductions of DA levels and elevation of dopamine turnover rate, as well as the ability of gastrodin to prevent alterations in D2 receptor and DAT protein levels. Together, it is suggested that gastrodin is a potential therapeutic intervention for IDPN-induced cognitive impairments.

Conflict of interest The authors declare that there are no conflicts of interest.

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