Journal of Natural Medicines
https://doi.org/10.1007/s11418-020-01451-4

NOTE

Diosgenin content is a novel criterion to assess memory enhancement effect of yam extracts

Ximeng Yang[1] · Kaori Nomoto[1] · Chihiro Tohda[1]

Received: 20 July 2020 / Accepted: 16 September 2020

Abstract
Several studies have suggested that some kind of *Dioscorea* species (yam) or yam-contained herbal medicines have cognitive enhancement effect. However, it has been unknown what is a crucial factor for cognitive enhancement in each *Dioscorea* species. In this study, we aimed to investigate whether one of the main and brain-penetrating components in yams, diosgenin, can be a novel criterion to assess memory enhancement effect of yam extracts. Although our previous studies showed that administration of diosgenin or diosgenin-rich yam extract enhanced cognitive function in normal mice and healthy humans, we have never evaluated whether the effect depends on diosgenin content or not. Therefore, we compared memory enhancement effects of low diosgenin-contained general yam water extract with diosgenin-rich yam extract on cognitive function in normal mice. We found that unlike diosgenin-rich yam, administration of general yam water extract did not enhance object recognition memory in normal mice. LC–MS/MS analyses revealed that after administration of general yam, diosgenin concentration in the brain did not reach to the effective dose because of the low diosgenin content in the original yam extract. On the other hand, when diosgenin was artificially added into general yam, the extract showed memory enhancement in normal mice and promoted neurite outgrowth in neurons. Our study suggests that diosgenin is actually an active compound in yams for memory enhancement, and diosgenin content can be a criterion for predicting cognitive enhancement effect of yam extracts.

Keywords *Dioscorea* · Yam · Diosgenin · Memory enhancement · Brain penetration · Neurite outgrowth

Introduction

Several studies have reported that the extracts from some kind of *Dioscorea* species (yam) have cognitive enhancement effect: *D. psedojaponica* Yamamoto and *D. opposita*; or yam-contained herbal medicine mixtures: traditional Japanese herbal medicine Hachimijiogan (containing *D. batatas*), US 7,273,626 B2 poly herbal formulation (containing *D. bulbifera*), and traditional Korean medicine mixture (containing *D. japonica*) [1–5]. These studies may show that *Dioscorea* species are widely beneficial for cognitive enhancement. However, it has been unknown what is a crucial factor for cognitive enhancement in each *Dioscorea* species.

✉ Chihiro Tohda
chihiro@inm.u-toyama.ac.jp

[1] Section of Neuromedical Science, Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan

We have previously reported that a steroid sapogenin, diosgenin, which is contained in several *Dioscorea* species, promotes axonal growth and regeneration, and enhances memory function in normal mice and Alzheimer's disease model mice [6, 7]. Diosgenin has been reported to have other several pharmacological activities such as anti-cancer, anti-diabetes, anti-food allergy, neuroprotective, and anti-inflammation effects [8–12]. We have also demonstrated that the mechanisms of diosgenin-induced axonal growth and memory enhancement were revealed to be mediated by the direct stimulation of a cell surface receptor, $1,25D_3$-MARRS (membrane-associated rapid response steroid-binding receptor) and the subsequent reduction of HSC70 in neurons [6, 7, 13, 14]. Furthermore, we for the first time reported that intake of diosgenin-rich yam extract enhances cognitive function in healthy humans [15]. And importantly, diosgenin is derived to the blood and brain after oral administrations when solved in some sort of oils [15].

Although our previous studies suggested that diosgenin may be an active component in yams for memory

Published online: 26 September 2020

enhancement, we have not evaluated that the memory enhancement effect of yams depends exclusively on diosgenin content. Diosgenin content is quite different in the species of *Dioscorea*. For example, *D. zingiberensis* and *D. collettii* contain rich amounts of diosgenin, while diosgenin contents in *D. japonica* and *D. opposita* is very low [16–18]. Dioscorea Rhizome defined in Japanese Pharmacopoeia standard is rhizome of *D. japonica* or *D. batatas* which contains very low amount of diosgenin. If only diosgenin is an active compound for cognitive enhancement in yam, water extract of the low diosgenin yam would not reveal enough effect on cognitive function.

In order to clarify whether diosgenin can be a criterion to assess memory enhancement effect of yam extracts, we used two different types of yam extracts: low diosgenin-contained general yam water extract (Japanese Pharmacopoeia standard Dioscorea Rhizome) and diosgenin-rich yam extract made by hydrolysis of diosgenin glycosides. The diosgenin-rich yam extract, which used in our previous clinical study, is made from Yunnan (China)-derived *D. batatas* which has higher diosgenin glycosides (> 2% in yam) than Japanese yam [15]. After obtaining the yam extract, it is then artificially treated with acid to increase diosgenin levels from the glycosides by acid hydrolysis. Therefore, diosgenin content is raised to 16% in this yam extract though this processing is quite different from the water extraction method used for general crude drugs.

In the present study, memory enhancement and neurite outgrowth effects of general yam water extract were compared with those of diosgenin-rich yam extract, and transfer of diosgenin to the brain was also compared. This study may also provide a template for discussing the crude drugs in terms of the importance of the contents of active compounds and contributions of active compounds into target tissues.

Materials and methods

Animal studies

All experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the University of Toyama. The Committee for Animal Care and Use at the Sugitani Campus of the University of Toyama approved the study protocols (approval number for the animal experiments is A2017INM-1). ddY mice were purchased from Japan SLC (Shizuoka, Japan). All efforts were made to minimize the number of animals used. All mice were housed in a controlled environment (25 ± 2 °C, 12 h light/dark cycle starting at 7:00 am) with free access to food and water.

Drug administration

The diosgenin-rich yam extract (Diopower 15®) was purchased from Anti-Aging Pro Corporation (Tokyo, Japan). This extract was prepared from *Dioscorea batatas*, and had an approximately 16% diosgenin content. The Japanese Pharmacopoeia-standard yam extract was prepared by Alps Pharmaceutical Ind. Co., Ltd (Gifu, Japan). 500 g Dioscorea Rhizome (15 mm pieces) were added into 5 L water, extracted at > 90 °C for 1 h, and dried it in powder (19.2% yield). Diosgenin (Fig. 1) compound was purchased from Tokyo Chemical Industry (Tokyo, Japan).

We dissolved 100 mg/kg yam, 100 mg/kg diosgenin-rich yam, or 100 mg/kg yam added with 16 mg/kg (38.59 μmol/kg) diosgenin in Japanese Pharmacopoeia quality standard olive oil (Maruishi Pharmaceutical Co., Ltd, Osaka, Japan). The extracts or vehicle solution (olive oil) was orally administered to the female ddY mice (6 weeks old) once a day for 7 days.

Novel object recognition test

On the next day after drug administrations, a novel object recognition test was performed. In the training session, each mouse was given two similar objects that were located at a fixed place within a square box. We recorded the number of times the mice made contact with the two objects within 10 min. After 48 h of the interval time, the test session was performed. In the test session, one of the objects used in the training session was replaced with a novel one (different shape and color with the familiar one). The number of times each mouse made contact with the familiar and novel objects was recorded within 10 min. The test session was conducted in a dimly illuminated room (44 lx).

Brain penetration of diosgenin

To calibrate the diosgenin content in each yam extracts, 100 μg/ml of each extract was dissolved in methanol (Fig. 3). To assess the blood–brain barrier (BBB) penetration of diosgenin after administration of the yam extracts, 100 mg/kg yam, 100 mg/kg diosgenin-rich yam, 16 mg/kg diosgenin, or vehicle solution (olive oil) was orally

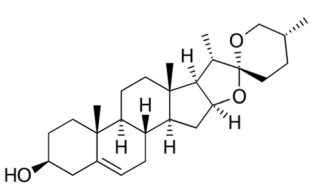

Fig. 1 Chemical structure of diosgenin is shown

administered to male ddY mice (7 weeks old) (Fig. 4, Fig. 5). Six, twenty-four, or forty-eight hours after drug administrations, the mice were euthanized and blood was collected. Plasma was obtained by centrifugation at 10,000 g for 10 min at 4 °C and extracted with methanol, dried, and resolubilized in 100 µl of methanol. The cerebral cortex perfused with saline before dissection was homogenized, extracted with methanol, dried, and resolubilized in 100 µl of methanol before LC–MS/MS analysis. To calculate the diosgenin concentration in the cortex, a standard curve was plotted. Briefly, standard solutions of diosgenin were extracted with methanol, and subjected to LC–MS analyses. A Thermo ScientificTM Accela HPLC system interfaced with an LTQ Orbitrap XL Hybrid Fourier Transform Mass Spectrometer (Thermo Fisher Co., San Jose, CA, USA) was used to chemically profile diosgenin. LC analyses were performed on a Capcell Pak C18 MGIII (2.0 mm i.d. × 150 mm, Shiseido, Tokyo, Japan) column held at 40 °C with a flow rate of 200 µl/min. Ultrapure water and methanol (M) were used as the mobile phase. The following linear elution gradient was used: 0–5 min, 65% M; 5–16 min, 95% M; 16–20 min, 55% M. The following electrospray ionization (ESI) parameters were used: spray voltage, 4.5 kV; capillary voltage, 40.0 kV; tube lens, 150 V; capillary temperature, 330 °C; sheath gas flow rate, 50 units; and aux gas flow rate, 10 units. We operated the mass spectrometer in the positive ESI mode; scanning from 50 to 2,000 m/z; and calibrated the instrument using a polytyrosine solution before each experiment.

Primary culture and immunocytochemistry

Embryos were removed from a pregnant ddY mouse at 14.5 days of gestation as previously described [6]. Cells were cultured for 3 days, followed by the addition of 2.5 µg/ml yam, 2.5 µg/ml diosgenin-rich yam, 2.5 µg/ml yam added with 0.4 µg/ml (1 µM) diosgenin, or vehicle solution (0.1% ethanol) for 4 days. The cells were fixed with 4% paraformaldehyde, and immunostained at 4 °C for 24 h using antibodies against the axonal marker, mouse phosphorylated neurofilament heavy subunit (pNF-H; monoclonal, 1:250, Covance, Princeton, NJ, USA), and against the neuronal and dendrite markers, rabbit microtubule-associated protein 2 (MAP2; polyclonal, 1:1000, Abcam, Cambridge, United Kingdom). Alexa Fluor 488-conjugated goat anti-mouse IgG (1:400) and Alexa Fluor 568-conjugated goat anti-rabbit IgG (1:400) were used as secondary antibodies. Fluorescence images (864.98 µm × 645.62 µm) were captured using a fluorescence microscopy system (Carl Zeiss, Oberkochen, Germany). The lengths of the pNF-H-positive axons and MAP2-positive dendrites were measured using MetaMorph version 7.8 (Molecular Devices, Sunnyvale, CA, USA).

Statistical analysis

Statistical comparisons were performed using one-way analysis of variance (ANOVA) with post hoc Dunnett's tests and repeated measures two-way ANOVA with the post hoc Bonferroni tests in GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). $p < 0.05$ was considered significant. Data are presented as the mean ± SEM.

Results

Diosgenin-rich yam, but not general yam, enhances cognitive function in normal mice

Previously, we reported that administration of diosgenin or diosgenin-rich yam extract for at least 4 days or 7 days, respectively, significantly enhanced object recognition memory in normal mice. Furthermore, the brain penetration of diosgenin was remarkably increased for olive oil-dissolved diosgenin or the extract compared with that dissolved in water [15]. Therefore, we compared memory enhancement effects of two yam extracts which dissolved in olive oil.

After administration of general yam water extract (Yam), diosgenin-rich yam (Dios-rich Yam), or vehicle solution (Veh; olive oil) for 7 days, a novel object recognition memory test was performed (Fig. 2). In the training session of this test, all the mice showed similar exploratory behaviors toward two identical objects (preferential indexes of approximately 50%). In the test session, the diosgenin-rich yam-treated mice showed a significantly higher preferential index to the novel object compared with the vehicle-treated mice. However, general yam-treated mice did not show enhancement of object recognition memory. These data indicate that only diosgenin-rich yam extract, but not general yam, is effective for memory enhancement in normal mice.

Detection of diosgenin contents in each yam extract

To evaluate the reasons why general yam water extract was not effective for memory enhancement, we first compared the diosgenin contents in both original yam extracts.

By detecting the high-accuracy quasi-molecular ion ($[M+H]^+$) with a mass error of ± 1 mmu, we could identify the standard mass data and fragmentation pattern of diosgenin (Fig. 3a). According to the mass spectrum of standard diosgenin (m/z = 415.3192, limit of detection; LOQ = 0.6 ng [0.06 µg/mL]), the diogsenin content in diosgenin-rich yam was detected. In general yam, no ion current peak

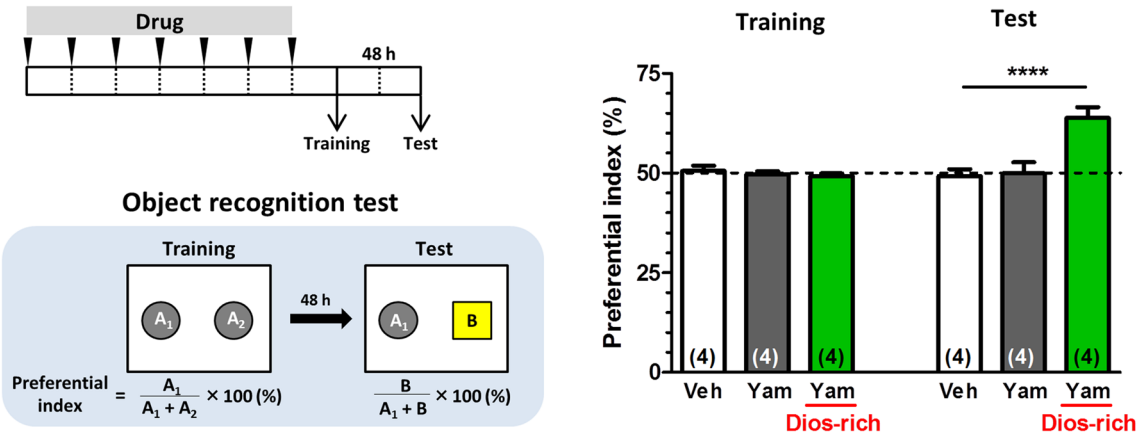

Fig. 2 Object recognition memory test was performed in each yam extract-administered mice. ddY mice (6 weeks old) were orally administered with general yam (Yam; 100 mg/kg/day), diosgenin-rich yam (Dios-rich Yam; 100 mg/kg/day), or vehicle solution (Veh; olive oil) for 7 days. After the drug administrations, a novel object recognition memory test was performed. The preferential indexes in the training and test sessions are shown. A significant drug × test interaction was found by repeated-measures two-way ANOVA [$F(2, 9) = 10.15$, $p = 0.0049$; $^{****}p < 0.0001$, post hoc Bonferroni test, $n = 4$]. Diosgenin-rich yam, but not general yam, enhanced object recognition memory in normal mice

was detected at a similar retention time to that of standard diosgenin. On the other hand, sharp diosgenin peaks were detected at the standard diosgenin retention time in the diosgenin-rich yam (Fig. 3b). These data indicate that there is almost no diosgenin (below LOQ) in general yam extract, and thus is possible the reason for general yam not showing memory enhancement.

Brain penetration of diosgenin after administration of each yam extract in mice

It is known that apart from diosgenin, diosgenin glycosides (ex: dioscin, protodioscin, pseudoprotodioscin) are also widely contained in *Dioscorea* species, which are acid hydrolyzed or biotransformed into diosgenin in vivo [19, 20]. Despite the very low diosgenin content in general yam, we investigated whether metabolized diosgenin after general yam extract administration would penetrate the brain.

To assess the brain penetration of diosgenin, similar doses of yam extract (100 mg/kg) as used in the behavior tests were orally administered to the mice. As a positive control, the same amount of diosgenin as that in the diosgenin-rich yam (16 mg/kg) was administered to the mice. Six hours after the administration, cortex and plasma samples were collected from the mice.

Based on the mass spectrum of standard diosgenin (m/z = 415.3196) (Fig. 4a), ion current peaks of diosgenin in the cortex samples obtained from each mouse were detected (Fig. 4b). In the cortex samples of diosgenin-rich yam- or diosgenin-administered mice, diosgenin peaks were detected at similar retention times as that of standard diosgenin, suggesting that diosgenin indeed penetrates the brain. Diosgenin was also detected in the plasma samples of the mice in these two groups (data not shown). On the other hand, corresponding diosgenin peaks were not detected (below LOQ) in the cortex and plasma samples of general yam- and vehicle-administered mice. Using standard curves of diosgenin, the predicted concentrations of diosgenin in the cortices of diosgenin-rich yam- and diosgenin-administered mice were quantified as 1.05 nmol/g and 0.09 nmol/g, respectively. These results suggested that only administration of diosgenin-rich yam extract enables sufficient penetration of diosgenin into the brain to possibly allow memory enhancement in mice.

On the other hand, administration of various *Dioscorea* such as *D. nipponica*, *D. panthaica*, and *D. zingiberensis*, has been reported to increase the plasma concentration of diosgenin at late time points (1–2 days) [20]. Although we used a different species in this study, we tried to collect the biosamples at late time points after extract administration. In addition, doses higher than the effective dose are sometimes used to detect compounds in biological samples by mass spectrometry [14, 21]. Therefore, 500 mg/kg general yam extract was orally administered to the mice, and cortex and plasma samples were collected at 24 or 48 h after administrations.

According to the mass spectrum of standard diosgenin (m/z = 415.3200) (Fig. 5a), ion current peaks of diosgenin in the cortex and plasma samples obtained from the mice were detected (Fig. 5b). On the other hand, no corresponding diosgenin peaks (below LOQ) were detected in the cortex and plasma samples obtained from general yam-administered mice. Since administration of higher extract doses is impractical when converted to human dose, we

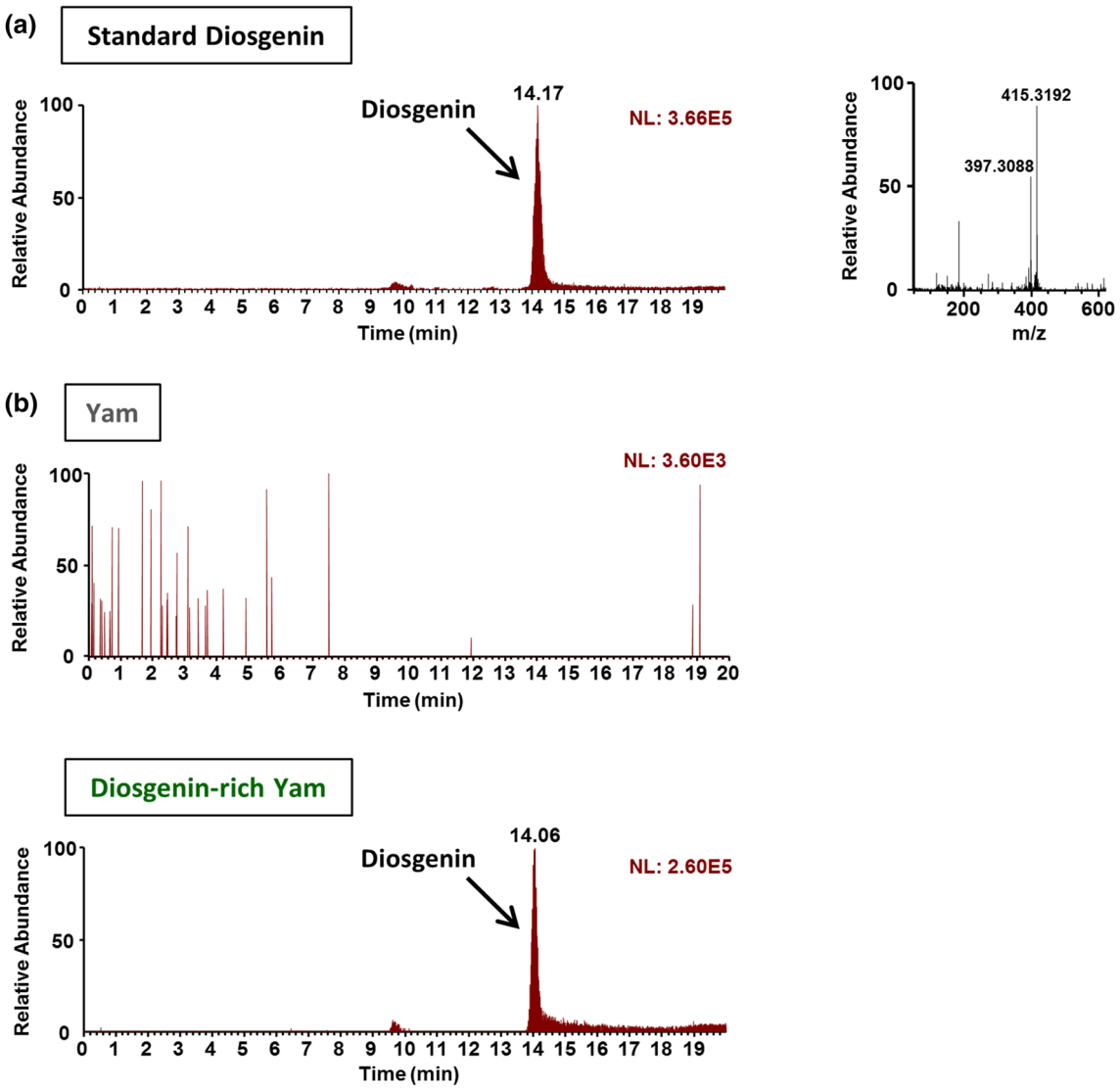

Fig. 3 The content of diosgenin was detected in each yam extract. **a** Standard peaks of diosgenin (10 μg/ml). Extracted ion current (EIC) chromatogram and mass spectrum (MS) of diosgenin (m/z = 415.3192) are shown (left and right panels, respectively). **b** 100 μg/ml yam or diosgenin-rich yam was dissolved in ethanol. EIC chromatograms show the contained diosgenin in each yam extract. Only diosgenin-rich yam extract showed high diosgenin content. NL: normalization level (base peak intensity)

concluded that the penetration of sufficient amount of diosgenin into the brain was not achieved after administration of general yam extract. This indicates that general yam water extract may not enhance cognitive function in normal mice.

Diosgenin is an active component in yams for memory enhancement and neurite outgrowth

Given the differences in the memory enhancement effect and diosgenin content of diosgenin-rich yam and general yam, we speculated that diosgenin is actually an active component in yams for memory enhancement. However, there should be many other natural compounds in diosgenin-rich yam extract made from *Dioscorea batatas*. To determine whether only improving the diosgenin content in general yam extract is sufficient to make it stimulate brain function, diosgenin was artificially added into the general yam water extract and its effect on memory function was assessed in normal mice.

After 7 days administration of 100 mg/kg general yam, 100 mg/kg general yam added with 16 mg/kg diosgenin (the same diosgenin content in diosgenin-rich yam), or vehicle solution (olive oil), a novel object recognition memory test was performed (Fig. 6). We found that the general yam + diosgenin-treated mice showed a significantly higher

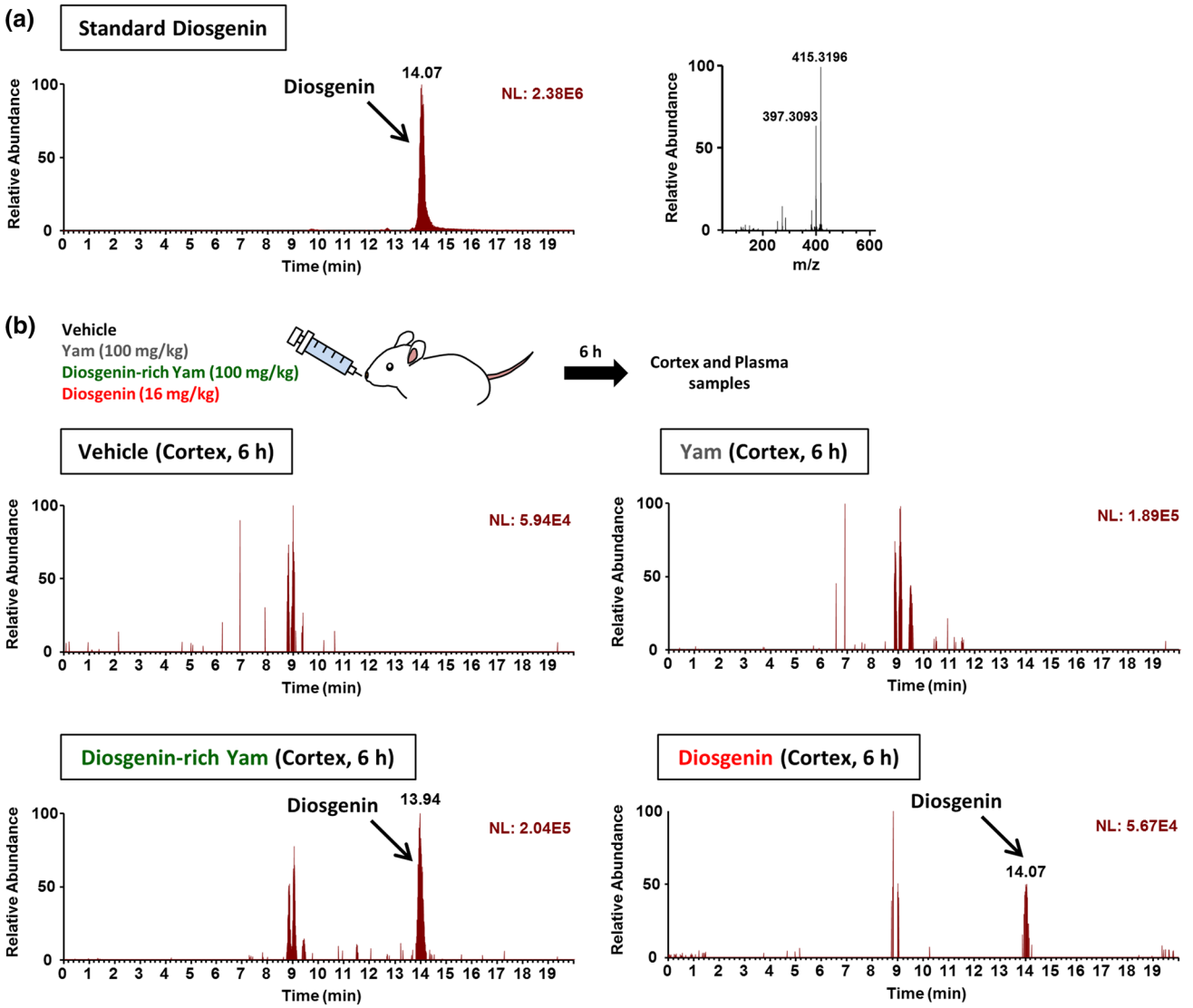

Fig. 4 Brain penetration of diosgenin after oral administration of each yam extract was investigated. **a** Standard peaks of diosgenin (20 μg/ml). Extracted ion current (EIC) chromatogram and mass spectrum (MS) of diosgenin (m/z = 415.3196) are shown (left and right panels, respectively). **b** ddY mice (7 weeks old) were orally administered with 100 mg/kg yam, 100 mg/kg diosgenin-rich yam, 16 mg/kg diosgenin, or vehicle solution (olive oil). Six hours after administrations, cortex samples were obtained. EIC chromatograms show the diosgenin content in each cortex sample. Diosgenin penetrated into the cortex only after administration of diosgenin-rich yam extract or diosgenin itself. NL: normalization level (base peak intensity)

preferential index to the novel object compared with the vehicle- or general yam-treated mice. These data indicate that high diosgenin content in yam extract is sufficient for memory enhancement in normal mice.

Next, we investigated whether the diosgenin content in yam extracts determines its effect on neurite outgrowth in primary cortical neurons. Previously, we reported that diosgenin treatment significantly promoted axonal growth and regeneration in primary neurons and the brains of mice [6, 7, 13]. In this study, based on our previous findings on significant axonal growth effect by the treatment of 1 μM diosgenin, we treated neurons with 2.5 μg/ml general yam extract, 2.5 μg/ml diosgenin-rich yam extract, or 2.5 μg/ml general yam added with 0.4 μg/ml (1 μM) diosgenin. We found that pNF-H-positive axons and MAP2-positive dendrites were not extended by general yam treatment; however, they were significantly extended by diosgenin-rich yam or general yam + diosgenin treatment (Fig. 7). These results showed in vitro evidence that high diosgenin content in yam extract is sufficient for stimulating neurite outgrowth and the active compound responsible for these effects is diosgenin.

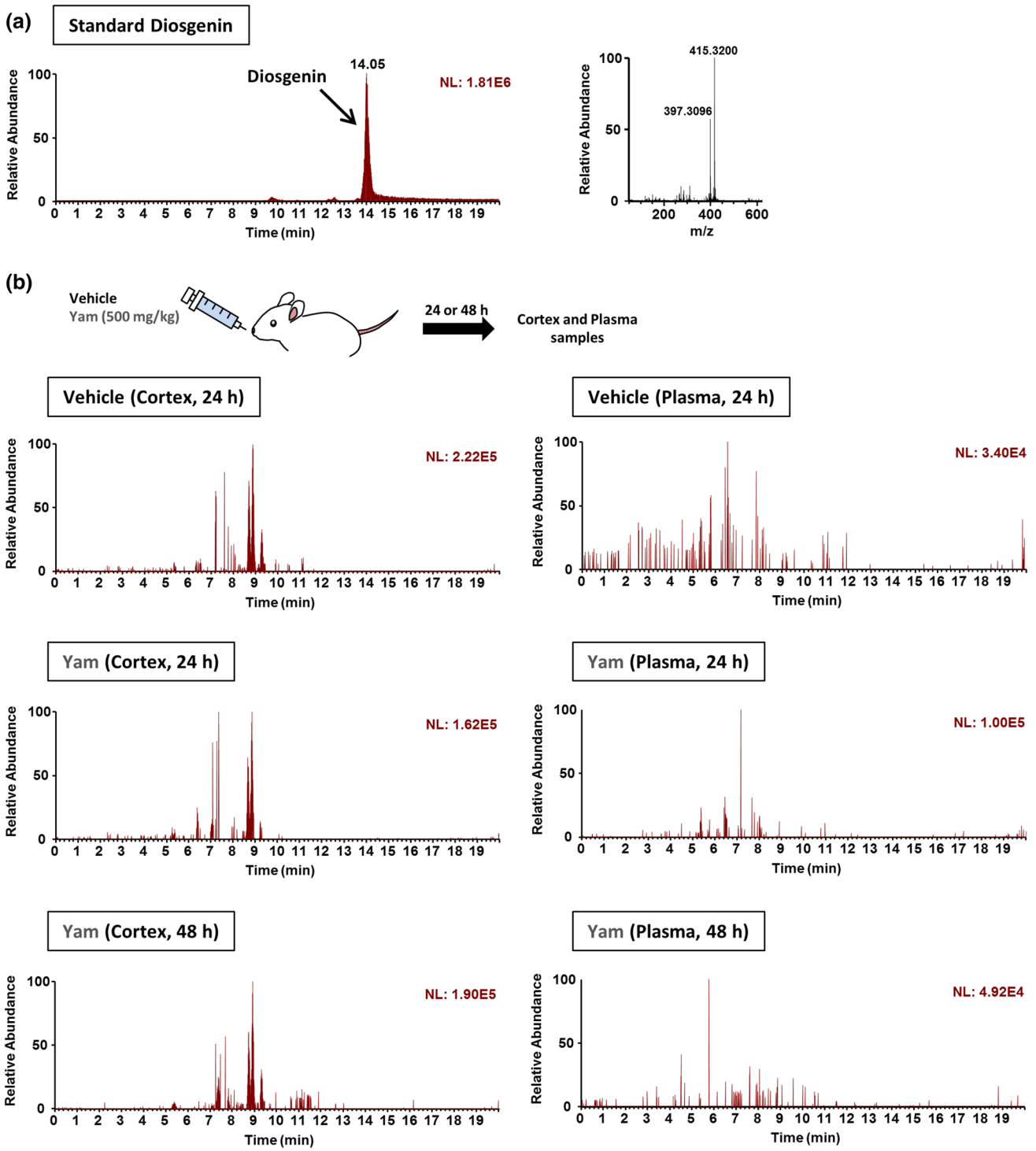

Fig. 5 Brain penetration of diosgenin after oral administration of high dose yam extract was investigated. **a** Standard peaks of diosgenin (10 μg/ml). Extracted ion current (EIC) chromatogram and mass spectrum (MS) of diosgenin (m/z = 415.3200) are shown (left and right panels, respectively). **b** ddY mice (7 weeks old) were orally administered with 500 mg/kg yam or vehicle solution (olive oil). Cortex and plasma samples were obtained 24 or 48 h after the administration. EIC chromatograms show the diosgenin content in each cortex sample. Diosgenin was not detected in the cortex and plasma samples even after administration of high dose of yam extract. NL: normalization level (base peak intensity)

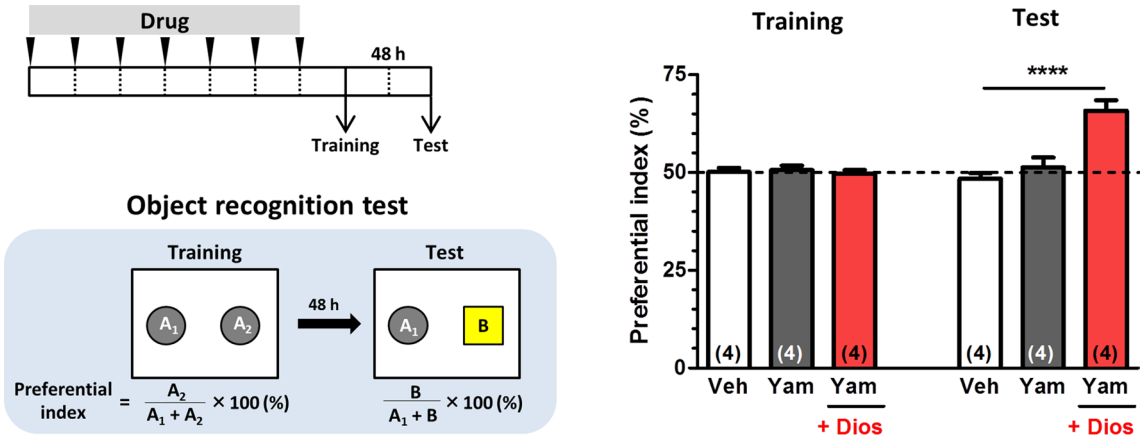

Fig. 6 Object recognition memory test was performed in diosgenin-added yam extract-administered mice. ddY mice (6 weeks old) were orally administered with 100 mg/kg general yam (Yam), 100 mg/kg yam added with 16 mg/kg diosgenin (Yam + Dios), or vehicle solution (Veh; olive oil) once a day for 7 days. After the drug administration, a novel object recognition memory test was performed. The preferential indexes in the training and test sessions are shown. A significant drug × test interaction was found by repeated-measures two-way ANOVA [$F(2, 9) = 11.94$, $p = 0.0029$; $^{****}p < 0.0001$, post hoc Bonferroni test, $n = 4$]. Diosgenin-added yam extract enhanced object recognition memory in normal mice

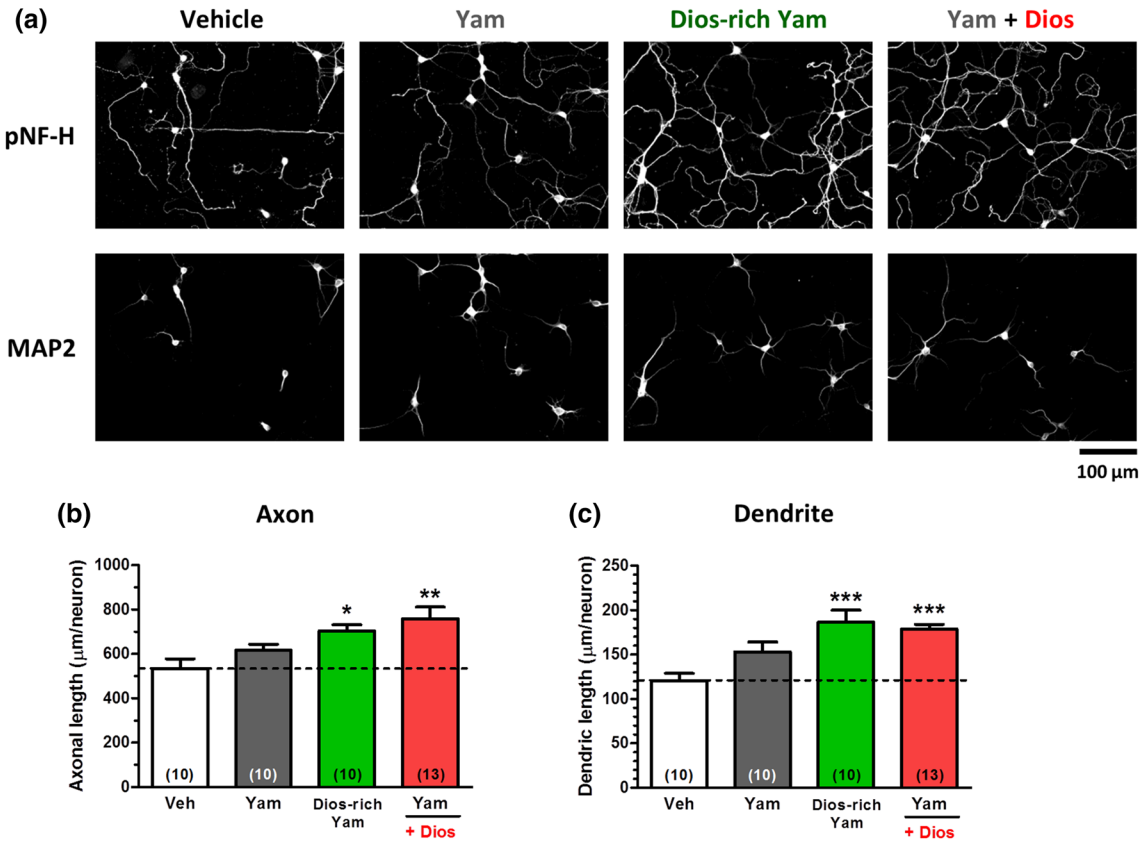

Fig. 7 Neurite outgrowth effects of each yam extract was investigated in primary cultured cortical neurons. Mouse cortical neurons (ddY, E14.5) were cultured for 3 days and then treated with 2.5 µg/ml general yam (Yam), 2.5 µg/ml diosgenin-rich yam (Dios-rich Yam), 2.5 µg/ml yam added with 1 µM diosgenin (Yam + Dios), or vehicle solution (0.1% EtOH) for 4 days. After the drug treatment, neurons were fixed and double-immunostained for phosphorylated neurofilament heavy subunit (pNF-H) and microtubule-associated protein 2 (MAP2). **a** Representative images of pNF-H-positive axons and MAP2-positive dendrites from each treatment are shown. **b** Lengths of pNF-H-positive axons and (C) MAP2-positive dendrites were quantified in each treatment group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs Veh, one-way ANOVA post hoc Dunnett's test. The number of measured areas is shown in each column

Discussion

The goal of this study was to evaluate whether memory enhancement effect of *Dioscorea* species depends on the diosgenin content in the extracts. To clarify this issue, we compared low diosgenin-contained general yam water extract and diosgenin-rich yam extract on memory function and neurite outgrowth effects. We found that unlike diosgenin-rich yam, administration of general yam water extract did not induce memory enhancement in normal mice and neurite outgrowth in cultured neurons. We attribute this to the low diosgenin content in the original extract, which does not allow the sufficient amount of diosgenin to penetrate into the brain and stimulate neurons directly. However, when diosgenin was added into the general yam, the extract showed memory enhancement and neurite outgrowth, suggesting that diosgenin is actually an active component for cognitive enhancement in yams, and high diosgenin content in yams is crucial to show its effectiveness on memory function.

Since diosgenin has been generally recognized as one of the main components in Dioscorea Rhizome, it was surprising that LC–MS/MS analyses did not detect any diosgenin in the original general yam extract at 100 μg/ml (Fig. 3). Although data are not shown, LC–MS/MS analyses did not detect diosgenin in up to 150 mg/ml of the general yam water extract. In addition, no diosgenin was detected in the plasma and brain even after administration of an excessive amount of general yam (Fig. 5), suggesting that the content of diosgenin glycosides were also very low in general yam. Other groups also reported that diosgenin was not detected in the methanol extract of Japanese Pharmacopoeia standard Dioscorea Rhizome, which is consistent with our findings [22]. Although we cannot discuss certainly without complete information about diosgenin contents in previous other studies used yam-contained extracts on memory enhancement, the effects might not be induced by diosgenin if they used low diosgenin-contained *Dioscorea* species [1–5].

Despite the finding that diosgenin content can be a criterion for predicting and evaluating cognitive enhancement effect of each yam extract, low diosgenin-contained yams still have some potential to exert effectiveness of diosgenin by increasing diosgenin content in extracts and tissue distribution of diosgenin. For example, one of the glycosides of diosgenin, dioscin, is widely contained in yams. Diosgenin needs acid hydrolysis (ex. gastric acid) to be biotransformed from dioscin [23]; however, it has low transformation efficiency in the body [24]. Therefore, if yams contain rich dioscin and other diosgenin glycosides just like Yunnan (China)-derived *D. batatas* used for diosgenin-rich yam in this study, diosgenin levels need to be increased from its glycosides by acid hydrolysis (Japan Patent Kokai 2017-6095591).

The bioavailability of diosgenin after administration is low despite the high hydrophobicity of diosgenin (cLogP = 5.912). Some groups have tried to improve the tissue distributions of diosgenin. Mixing β-cyclodextrin with diosgenin has been reported to enhance its skin distribution [23]. In our previous studies, the plasma concentration and brain penetration of diosgenin after oral administration were enhanced by dissolving diosgenin in several kinds of oil solvents. While 10 μmol/kg/day diosgenin was only effective when administrated intraperitoneally, but not for orally, when dissolved in 10% ethanol + 5% glucose aqueous solution. In contrast, oral administration of 0.1 μmol/kg/day diosgenin significantly enhanced memory function when sesame, olive, or soybean oil was used as solvents. Furthermore, diosgenin-rich yam extract suspended in olive oil gave long period accumulation of diosgenin in the brain compared with that in water [15].

Among various kinds of Dioscorea species, we chose Japanese Pharmacopoeia standard quality yam derived from *D. japonica* or *D. batatas* as a representation for low diosgenin-contained yam in this study. This was also due to our interest that whether generally used yam in Japan has memory enhancement effect or not. Therefore, further investigations should be conducted using other species or origin of low diosgenin-contained yams to support this finding. Furthermore, the relationships between diosgenin content in yams and its pharmacological activity on memory enhancement effect should be clarified using several different amounts of diosgenin-contained yams.

This is the first study to demonstrate that unlike diosgenin-rich yam, administration of low diosgenin-contained general yam water extract does not exert the diosgenin effect on memory enhancement. We attributed this to be insufficient amount of diosgenin reaches to the brain after administration because of the low diosgenin content in the original yam extract. Therefore, it was indicated that diosgenin is actually an active component in yams for memory enhancement, suggesting a novel criterion to assess cognitive enhancement effect of each yam extract.

Acknowledgements This research was funded by a grant-in-aid for the Cooperative Research Project from the Institute of Natural Medicine at the University of Toyama in 2017, and the Discretionary Funds of the President of the University of Toyama. We would like to thank Editage (www.editage.jp) for English language editing. We would like to thank the support of 2019 Director Leadership Expenses, Institute of Natural Medicine, University of Toyama.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest associated with this manuscript.

References

1. Chiu CS, Deng JS, Hsieh MT, Fan MJ, Lee MM, Chueh FS, Han CK, Lin YC, Peng WH (2009) Yam (*Dioscorea pseudojaponica* Yamamoto) ameliorates cognition deficit and attenuates oxidative damage in senescent mice induced by D-galactose. Am J Chin Med 37:889–902
2. Yang MH, Yoon KD, Chin YW, Park JH, Kim SH, Kim YC, Kim J (2009) Neuroprotective effects of *Dioscorea opposita* on scopolamine-induced memory impairment in in vivo behavioral tests and in vitro assays. J Ethnopharmacol 121:130–134
3. Kubota K, Fukue H, Sato H, Hashimoto K, Fujikane A, Moriyama H, Watanabe T, Katsurabayashi S, Kainuma M, Iwasaki K (2017) The traditional japanese herbal medicine hachimijiogan elicits neurite outgrowth effects in PC12 cells and improves cognitive in AD model rats via phosphorylation of CREB. Front Pharmacol 8:850
4. Upadhyay P, Sadhu A, Singh PK, Agrawal A, Ilango K, Purohit S, Dubey GP (2018) Revalidation of the neuroprotective effects of a United States patented polyherbal formulation on scopolamine induced learning and memory impairment in rats. Biomed Pharmacother 97:1046–1052
5. Jeon S, Lee CH, Liu QF, Kim GW, Koo BS, Pak SC (2014) Alteration in brain-derived neurotrophic factor (BDNF) after treatment of mice with herbal mixture containing *Euphoria longana.* Houttuynia cordata and Dioscorea japonica. Daru 22:77
6. Tohda C, Urano T, Umezaki M, Nemere I, Kuboyama T (2012) Diosgenin is an exogenous activator of $1,25D_3$-MARRS/Pdia3/ERp57 and improves Alzheimer's disease pathologies in 5XFAD mice. Sci Rep 2:535
7. Tohda C, Lee YA, Goto Y, Nemere I (2013) Diosgenin-induced cognitive enhancement in normal mice is mediated by $1,25D_3$-MARRS. Sci Rep 3:3395
8. Yan LL, Zhang YJ, Gao WY, Man SL, Wang Y (2009) In vitro and in vivo anticancer activity of steroid saponins of Paris polyphylla var. yunnanensis. Exp Oncol 31:27–32
9. Sangeetha MK, ShriShri Mal N, Atmaja K, Sali VK, Vasanthi HR (2013) PPAR's and Diosgenin a chemico biological insight in NIDDM. Chem Biol Interact 206:403–410
10. Huang CH, Ku CY, Jan TR (2009) Diosgenin attenuates allergen-induced intestinal inflammation and IgE production in a murine model of food allergy. Planta Med 75:1300–1305
11. Chen XB, Wang ZL, Yang QY, Zhao FY, Qin XL, Tang XE, Du JL, Chen ZH, Zhang K, Huang FJ (2018) Diosgenin glucoside protects against spinal cord injury by regulating autophagy and alleviating apoptosis. Int J Mol Sci 19:2274
12. Wang S, Wang F, Yang H, Li R, Guo H, Hu L (2017) Diosgenin glucoside provides neuroprotection by regulating microglial M1 polarization. Int Immunopharmacol 50:22–29
13. Yang X, Tohda C (2018) Diosgenin restores Aβ-induced axonal degeneration by reducing the expression of heat shock cognate 70 (HSC70). Sci Rep 8:11707
14. Yang X, Tohda C (2018) Heat shock cognate 70 inhibitor, VER-155008, reduces memory deficits and axonal degeneration in a mouse model of alzheimer's disease. Front Pharmacol 9:48
15. Tohda C, Yang X, Matsui M, Inada Y, Kadomoto E, Nakada S, Watari H, Shibahara N (2017) Diosgenin-rich yam extract enhances cognitive function: a placebo-controlled, randomized, double-blind. Crossover study of healthy adults. Nutrients 9:1160
16. Yi T, Fan LL, Chen HL, Zhu GY, Suen HM, Tang YN, Zhu L, Chu C, Zhao ZZ, Chen HB (2014) Comparative analysis of diosgenin in Dioscorea species and related medicinal plants by UPLC-DAD-MS. BMC Biochem 15:19
17. Vendl O, Wawrosch C, Noe C, Molina C, Kahl G, Kopp B (2006) Diosgenin contents and DNA fingerprint screening of various yam (*Dioscorea sp.*) genotypes. Z Naturforsch C 61:847–855
18. Li J, Yang D, Yu K, He J, Zhang Y (2010) Determination of diosgenin content in medicinal plants with enzyme-linked immunosorbent assay. Planta Med 76:1915–1920
19. Dong J, Lei C, Lu D, Wang Y (2015) Direct Biotransformation of Dioscin into Diosgenin in Rhizome of *Dioscorea zingiberensis* by Penicillium dioscin. Indian J Microbiol 55:200–206
20. Tang YN, Pang YX, He XC, Zhang YZ, Zhang JY, Zhao ZZ, Yi T, Chen HB (2015) UPLC-QTOF-MS identification of metabolites in rat biosamples after oral administration of Dioscorea saponins: a comparative study. J Ethnopharmacol 165:127–140
21. Yang Z, Kuboyama T, Tohda C (2017) A systematic strategy for discovering a therapeutic drug for alzheimer's disease and its target molecule. Front Pharmacol 8:340
22. Kawazoe S, Hashiguchi M, Tanaka R, Okumura A, Terabayashi S (2017) Contents of diosgenin and dioscin in wild yam supplements. J Food Sci 72:25–31
23. Jesus M, Martins AP, Gallardo E, Silvestre S (2016) Diosgenin: recent highlights on pharmacology and analytical methodology. J Anal Methods Chem 2016:4156293
24. Okawara M, Tokudome Y, Todo H, Sugibayashi K, Hashimoto F (2013) Enhancement of diosgenin distribution in the skin by cyclodextrin complexation following oral administration. Biol Pharm Bull 36:36–40