Toxicology Reports 14 (2025) 101904

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Neuronal effect of 0.3 % DMSO and the synergism between 0.3 % DMSO and loss function of UCH-L1 on *Drosophila melanogaster* model

Mai Thi Thu Trinh [a,b], Truong Huynh Kim Thoa [a,b], Dang Thi Phuong Thao [a,b,*]

[a] Laboratory of Molecular Biotechnology, University of Science-VNU.HCM, Viet Nam
[b] Department of Molecular and Environmental Biotechnology, Faculty of Biology -Biotechnology, University of Science-VNU.HCM, Viet Nam

ARTICLE INFO

Handling Editor: Dr. L.H. Lash

Keywords:
Active zone
DMSO
Drosophila melanogaster
Glutamate receptor
Synapse

ABSTRACT

Dimethyl sulfoxide (DMSO) is a polar aprotic solvent which is widely used in biological and medical studies and as a vehicle for pharmacological therapy. DMSO from 0.1 % to 0.5 %, particularly 0.3 % is commonly used as solvent to dissolve compounds when testing their effect on living cell, tissues including nerve cell. However, scientific data on the effects of DMSO on nervous system is limited. Here, we present our data of case study on investigation the effects of DMSO at 0.3 % concentration on nerve cell of *Drosophila melanogaster* model. We found that 0.3 % DMSO concentration had affected on the active zone and glutamate receptor. Notably, this study also revealed the synergistic effect of 0.3 % DMSO and loss function of dUCH (the homolog of Ubiquitin Carboxyl terminal Hydrolase -L1, UCH-L1 in *D. melanogaster*). This combination caused more serious abnormalities in synapse structure, particularly number of boutons on Neuromuscular Junction, NMJ. Furthermore, 0.3 % DMSO reduced the amount of ubiquitinylated protein aggregates in the indirect flight muscle of both normal and genectic defect fly model. Taken together, data in this sytudy indicated that 0.3 % DMSO caused the aberrant morphology of the synaptic structure and decreased the number of ubiquitinylated proteins in the indirect flight muscle of *Drosophila*. The data from the study contributed new evidence of the effects of DMSO on the nervous system. Signigicantly, this study revealed that DMSO affected on neuron cell at low concentration which widely used as pharmacological solvent.

1. Introduction

Dimethyl sulfoxide (DMSO) is an amphiphilic compound with two hydrophobic methyl groups and one hydrophilic sulfoxide group. Therefore, DMSO is the miscibility of water and able to dissolve lipophilic compounds make it a solvent in biological studies and an appreciated solvent for drug therapy [28]. DMSO has been used successfully in many human therapeutic situations [23,26,29]. In 1978, the United States Food and Drug Administration (FDA) approved its usage for use in the treatment of interstitial cystitis base on anti-inflammatory and analgesic properties [35]. Besides that, DMSO also has antioxidant properties; thus, its use has been in the treatment of several neurodegenerative disorders, such as Alzheimer's disease [36] and traumatic brain injury [12]. In 1992, DeForge et al. observed that 1 % DMSO concentration inhibited IL-8 expression at the level of transcription and this effect is related to hydroxyl radicals (•OH) scavengers action of DMSO [11]. Moreover, DMSO has been used effectively in the treatment of traumatic brain edema because it can crosse the blood-brain barrier [5]. On the other hand, there are some reports about the side effects of DMSO in biological studies. Research on EL-4 lymphoma cells showed that 2.5 % DMSO induced apoptosis through the release of cytochrome c, activating caspase 9, caspase 3 and down-regulation of Bcl-2 [25]. Importantly, DMSO at different concentrations and durations of use can cause damage and toxicity to the nervous system. DMSO dose of 0.5 % and 1 % produced neuronal loss in an in vitro rat hippocampal culture preparation [15]. Besides, DMSO at concentrations of 1 % caused mitochondrial damage, membrane potential impairment and reactive oxygen species production, as well as decreased cell viability and glial glutamate transporter expression in cultured astrocytes for 24 h [45]. Concurrently, DMSO dose 5 % significantly inhibited cell variability, increased apoptosis of astrocytes [45]. Study of G. Cavalentii et al. (2000) observed that 1.8 % and 3.6 % DMSO concentrations injected into the peritoneum of rats for 10 consecutive days did not affect the structure of the peripheral nervous system [8]. However, DMSO 7.2 % had the effect of changing the structure of the sciatic nerve with myelin disruption and uncompacted myelin lamellae [8]. Noticeably, even

* Corresponding author at: Laboratory of Molecular Biotechnology, University of Science-VNU.HCM, Viet Nam.
E-mail address: thaodp@hcmus.edu.vn (D. Thi Phuong Thao).

https://doi.org/10.1016/j.toxrep.2025.101904
Received 16 November 2024; Received in revised form 27 December 2024; Accepted 7 January 2025
Available online 8 January 2025

extremely low concentrations (0.05 %) of DMSO decreased both the input resistance of hippocampal neurons and excitability [1]. Nevertheless, the effect and mechanism of DMSO on the nervous system are limited and unclear. Therefore, studying the effects of DMSO on the nervous system at specific concentrations and models is necessary to provide information for the use of DMSO in biomedical research and disease treatment.

Neuromuscular junction (NMJ) is a synapse between a motor neuron and a muscle. The structure of NMJ comprises a presynaptic terminal (terminal bouton), which contains synaptic vesicles filled with the neurotransmitter (synaptic active zones); synaptic cleft, where neurotransmitter is released and postsynaptic receptor region on the muscle [4]. NMJ stability is crucial condition that needed for an efficient and reliable signals transmission from the motor neuron to the muscle in order to achieve the desired movement [17]. The NMJ stability can be assessed based on structural parameters such as number and size of boutons, active zones, and postsynaptic receptors [7]. Abnormal structure and dysfunction of NMJ caused neurodegenerative diseases and thereby, NMJ is the site of action for many pharmacological drugs [9, 43].

Ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) is a protein highly expressed in the brain and has been linked to neurodegenerative disorders [33]. Remarkably, exposure to pesticides such as rotenone and paraquat, as well as heavy metals, may interact with UCH-L1 dysfunction to accelerate neuronal damage in Parkinson's disease [40]. Besides that, UCH-L1 was also reported as an important protein that functions in synapse [6]. In *Drosophila melanogaster*, dUCH (Drosophila ubiquitin carboxyl-terminal hydrolase) presents as human UCH-L1 with 75.7 % similarity, 44.5 % identity [41]. Specific knockdown of dUCH (a homolog of the human UCHL-1) in motor neurons of *Drosophila melanogaster* caused aberrant morphology of synapses and decreased the density of the synaptic active zone and glutamate receptor area at the NMJ [20].

Drosophila melanogaster has been used as an in vivo model organism for the study of genetics and development, particularly the field of neuroscience, for a century [3]. Recently, *D. melanogaster* is widely used as a model organism in toxicology studies due to its unique advantages. These include genetic similarity to humans, short life cycle and high reproductive rate, cost effectiveness and ethical advantages [22]. Moreover, *D. melanogaster* offers various well-characterized organ and system models (e.g., nervous, cardiovascular, and digestive systems) for studying organ-specific toxicity. For instance, the fly's brain and gut are used to model neurotoxicity and intestinal toxicity [37,42]. According to N. Az mi et al. (2003), DMSO at 0.3 % (v/v) is a NOAEL (No Observed Adverse Effect Level) value that can be utilized as a dietary concentration in *D. melanogaster* toxicity investigations [10,32]. In the present study, we present experimental data which showed the effects of 0.3 % DMSO on *Drosophila* neurons.

2. Materials and methods

2.1. Fly stocks and food preparation

Fly stocks were maintained on standard food containing 0.8 % agar (Agar, TypeI; HiMedia), 5 % sucrose (food sugar, local brand), 5 % yeast extract (Saf-Instant Yeast), and 3 % powdered milk (full cream milk powder, local brand), propionic acid 0.5 % (v/v; 800605; Merck), and sodium benzoate 0.1 % (w/v; food additives, local brand), at 28 °C. DMSO was added to standard food at a final concentration of 0.3 % DMSO (v/v; 1.029.521.000; Merk). After mating, fly embryos were collected every 4 h and added to the experience medium at a density of approximately 70 embryos per 4 mL of medium per 1 vial, maintained at a constant temperature of 28 °C. Following eclosion, transfer the emerged adult flies to a new medium every 48 h. The medium was prepared just before usage.

We generated a motor neuron-specific Drosophila Ubiquitin Carboxyl Hydrolase (dUCH) knockdown fly strain by crossing the driver strain, which expresses GAL4 protein in motor neurons, D42-GAL4 (8816, Bloomington Drosophila Stock Center - BDSC), and the RNAi line carrying UAS-dUCH.IR (v26468, Vienna Drosophila Resource Center - VDRC). Control fly strain was generated by crossing the D42 driver with the wildtype Canton-S (BDSC).

2.2. Immunostaining

In these experiments, the neuromuscular junction of third-instar larvae was dissected in HL3 saline.

For Discs-large (DLG) immunostaining, the tissue was fixed with 4 % paraformaldehyde in PBS for 30 min at 25 °C, then washed with PBS containing 0.3 % Triton X-100 (PBST 0.3 %,), blocked with PBS containing 0.15 % Triton X-100 (0.15 % PBST) and 10 % normal goat serum (NGS). For BRP and GluR immunostaining, the tissue was fixed with Bouin's solution (Sigma-Aldrich, HT10132) at 25 °C for 10 min, then washed with 0.3 % PBST, and blocked with 0.27 % PBST containing 0.5 % of Bovine serum albumin (BSA). All the primary antibodies were diluted in blocking solution, respectively, then incubated the tissue for 16 h at 4 °C. The primary antibodies were used: mouse anti-Discs large (DLG) IgG (1:300, Developmental Studies Hybridoma Bank (DSHB), 4F3), mouse anti-Bruchpilot (BRP) IgG (1:200, DSHB nc82), and mouse anti-Glutamate Receptor IIA (GluR) IgG (1:80, DSHB 8B4D2). After washing with 0.3 % PBST, the tissue was incubated with secondary antibodies conjugated with Alexa 594 (1:400, Invitrogen) and FITC-conjugated goat anti-HRP IgG (1:1000, MP Biochemicals) at 25 °C for 2 h, washed again with PBST 0.3 % and mounted with Vectashield Mounting Medium (Vector Laboratories, Japan).

To evaluate protein aggregates, the indirect flight muscle of *Drosophila* at 15 days old was dissected and fixed as previously described [34], and then incubated with mouse anti-mono- and poly-ubiquitinylated conjugate IgG (1:300, Enzo Life Sciences, NY, USA) for 16 h at 4 °C. The samples were then rinsed with 0.3 % PBST, incubated with Alexa 594 anti-mouse IgG (1:400, Invitrogen) for 2 h at 25 °C, then washed again with 0.3 % PBST and mounted with Vectashield Mounting Medium (Vector Laboratories, Japan).

Finally, the samples were inspected using a Nikon fluorescence microscopy (Ni-U, Nikon, Japan), Confocal laser scanning microscope (Olympus Fluoview FV10i) or super resolution microscope (N-SIM, Nikon). The collected images were analyzed with the ImageJ software.

2.3. Data analysis

Microsoft Excel was utilized for data collection, GraphPad Prism 9.0 (GraphPad Software, USA) was used to analyze the statistics of all results. The figure legend describes the particular statistic test was used in each experiment.

3. Results

3.1. 0.3 % DMSO affected on the active zone area at the neuromuscular junction

The active zones are specialized areas on the presynaptic membrane of nerve cells, plays important role on neurotransmitter release [4]. To investigate the effects of DMSO on active zones, we used 0.3 % DMSO medium to feed the fly and collect the fly at developmental stage (larvae stage) and found that the larvae fed by the DMSO containing medium had a reduction on active zone areas. Particularly, comparison to non-treated larvae, the ratio of active zone area per synaptic area in the DMSO treated larvae reduced by 16 % in normal fly ($p = 0.0241$ – Fig. 1B).

To address if 0.3 % DMSO cause any change in the genetic defect fly, the loss function of *Drosophila* Ubiquitin Hydrolase (dUCH) – a homolog of Ubiquitin Carboxyl Hydrolase -L1 (UCH-L1) was utilized. We found

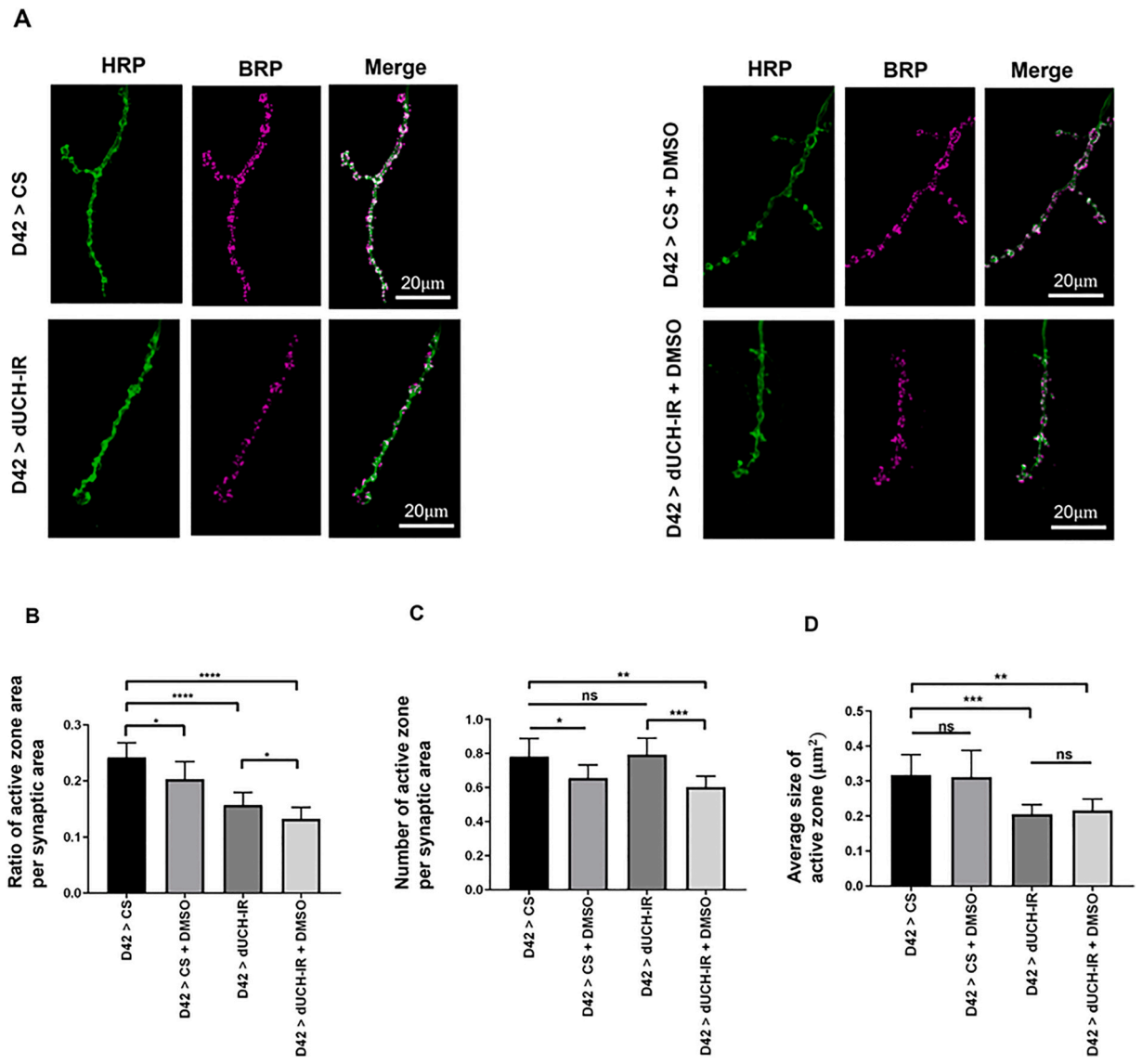

Fig. 1. 0.3 % DMSO affected on the active zone area at the neuromuscular junction (NMJ). (A) Double-stained 4th muscle NMJ with anti-HRP IgG (*green*) and anti-BRP IgG (*magenta*). Scale bars: 20 μm. Normal fly: D42 > CS, w/Y; + ; D42-GAL4/+ ; genetic defect fly model: D42 > dUCH-IR, w/Y; + ; D42-GAL4/UAS-dUCH IR. (B) Quantified data for the ratio of active zone area per synaptic area ($\mu m^2/\mu m^2$). (C) Quantified data for the ratio of active zone number per synaptic area (number/μm^2). (D) Quantified data of active zone average size (μm^2). Error bars indicate mean ± SD. Two-tail Student's *t*-test, *p < 0.05, **p < 0.01, ****p < 0.0001, n = 7.

that 0.3 % DMSO decreased the ratio of active zone area per synaptic area in the genetic defect fly model (p = 0.0470 – Fig. 1B). Reduction on the ratio of active zone area per synaptic area given by dUCH loss function (kdUCH) was 35 % (p < 0.0001) while that of combination of DMSO and dUCH loss function (DMSO/kdUCH) was 45 % (p < 0.0001). Besides, 0.3 % DMSO treatment decreased the number of active zone per synaptic area by 16 % (p = 0.0248) in normal larvae and 24 % (p = 0.0008) in kdUCH larvae (Fig. 1 C). The 0.3 % DMSO treatment caused no significant change on average size of active zone in both normal and genetic defect fly model compared to that of corresponding DMSO non-treated flies (Fig. 1D).

3.2. 0.3 % DMSO affected on the synapse structure formation at the neuromuscular junctions

The *Drosophila* larval neuromuscular junction (NMJ) has a structure consisting of many branches, each branch forms multiple swellings called synaptic boutons. The presynaptic bouton contains the active zones where neurotransmitter releasing [27]. There are two major types of glutamatergic motor neurons: Ib (big) and Is (small), which have different structural and physiological characteristics at the synaptic level at the NMJ. Type Ib boutons are surrounded by more subsynaptic reticulum (SSR) membrane than the type Is boutons [24]. To examine the effect of DMSO on NMJ, we focused on analyzing the NMJ branch length, the number, and the size of Ib boutons after larval feeding on 0.3 % DMSO containing medium. In the normal larvae, 0.3 % DMSO did not change the NMJ branch length, number and size of Ib boutons. Knockdown dUCH resulted in abnormal NMJ morphometry through reducing the NMJ branch length by 25 % (p = 0.0021), increasing the size of boutons by 34 % (p = 0.0001) and decreasing the number of boutons by 22 % (p = 0.0004) compared with normal larvae. Notably, the results indicated a synergism of 0.3 % DMSO and the loss function of dUCH in which number of boutons was stronger decreased by 29 % (p = 0.0004) compared to that of non-treated normal flies. These results could mimic the interaction between environmental factor (0.3 % DMSO) and genetic factor (loss function of dUCH) on the morphology of NMJ.

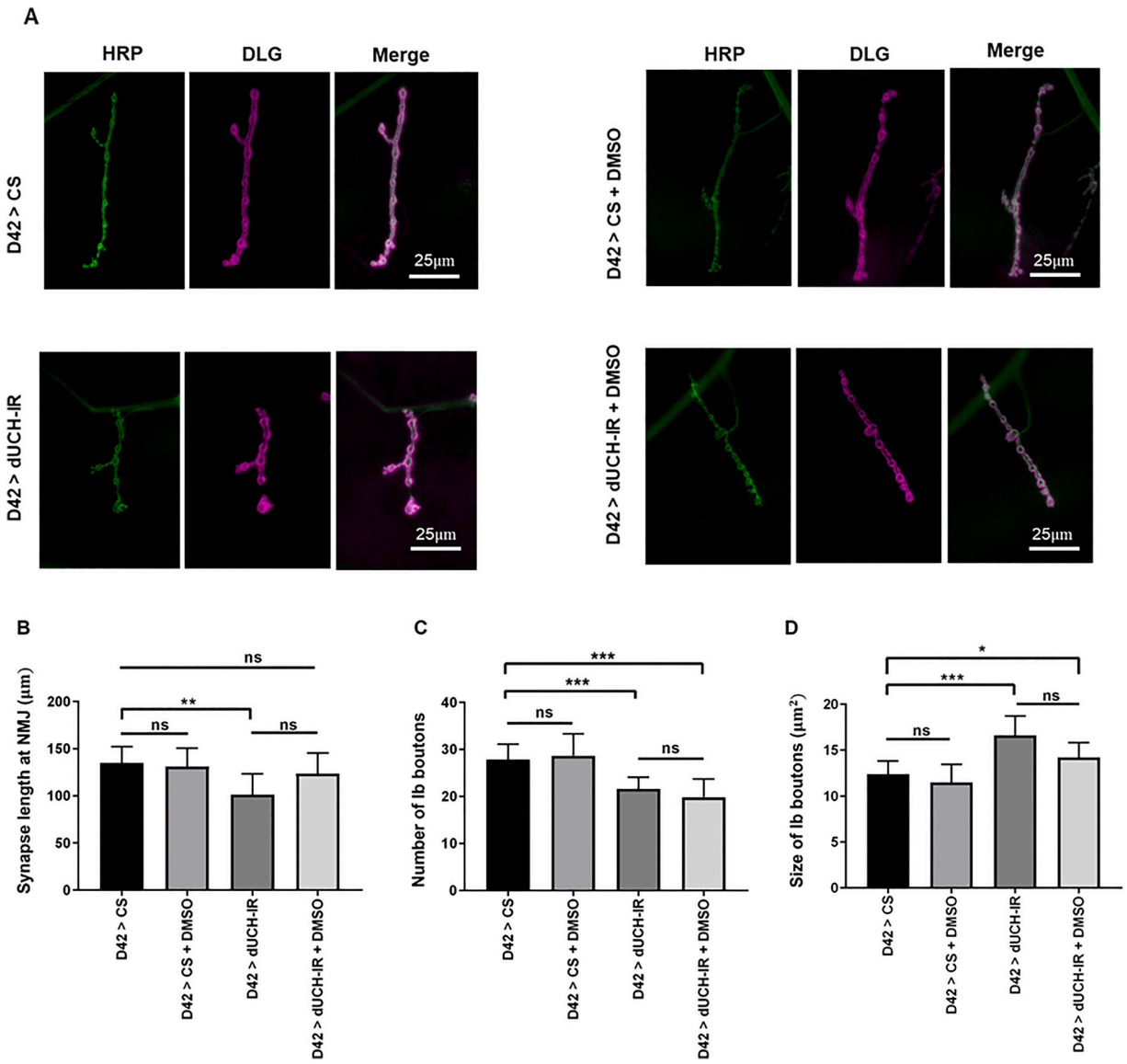

Fig. 2. 0.3 % DMSO affected structure of synapse at the neuromuscular junction. (A) Double-stained 4th muscle NMJ with anti-HRP IgG (*green*) and anti-DLG IgG (*magenta*). Scale bars: 25 µm. Normal fly: D42 > CS, w/Y; + ; D42-GAL4/+ ; genetic defect fly model: D42 > dUCH-IR, w/Y; + ; D42-GAL4/UAS-dUCH IR. (B) Quantified data of synapse length at NMJ. (C) Quantified data for number of Ib boutons. (D) Quantified data for size of Ib boutons. (μm^2). Error bars indicate mean ± SD. Two-tail Student's *t*-test, *p < 0.05, **p < 0.01, ****p < 0.0001, n = 6–10.

3.3. 0.3 % DMSO affected on the glutamate receptors of the NMJ

Glutamate receptors (GluRs) are important neuromodulators, responsible for excitatory neurotransmitter. GluRs received glutamate signaling at muscle cells, opposed to active zone sites [2]. In this study, we found that 0.3 % DMSO decreased the glutamate receptor density by 17 % ($p = 0.0038$) in normal larvae while it caused no significantly different on that of dUCH loss-function larvae. The loss-function of dUCH decreased glutamate receptor density by 27 % ($p = 0.001$) compared to that of fly with normal dUCH level. Interestingly, the combination of dUCH loss function and DMSO had just decreased the glutamate receptor density by 14 % ($p = 0.0232$) compared to the normal function of dUCH larvae feeding on normal medium. The results sugessted an interaction of environmental and genetic factors on the affects of glutamate receptor density.

3.4. 0.3 % DMSO decreased the amount of protein aggregates in the indirect flight muscle

Muscle function and physiology are dependent on appropriate innervation by motor neurons. Successful innervation includes the formation of synapses that comprise the NMJ [20,21]. In *D. melanogaster*, one of the tissues that receives signals from motor neurons is the indirect flight muscle, being the largest muscle and a model to research aging muscle development and disease [14]. Since aging and muscle degeneration are reported as factors which involved in signals transmission between motor neurons [17]. Besides that, previous research has demonstrated that the accumulation of ubiquitinylated proteins is a marker of muscle aging and degeneration [19]. In this study, we evaluated the amount of protein aggregates in the indirect flight muscle of *Drosophila* at 15 days old under impact of 0.3 % DMSO. Our results showed that 0.3 % DMSO caused the reduction on level of ubiquitinylated proteins in both normal and dUCH loss-function fly by 46 % ($p = 0.015$) and 38 % ($p = 0.0052$), respectively. Thereby, while loss-function of dUCH increased in the ubiquitinylated proteins by 68 %

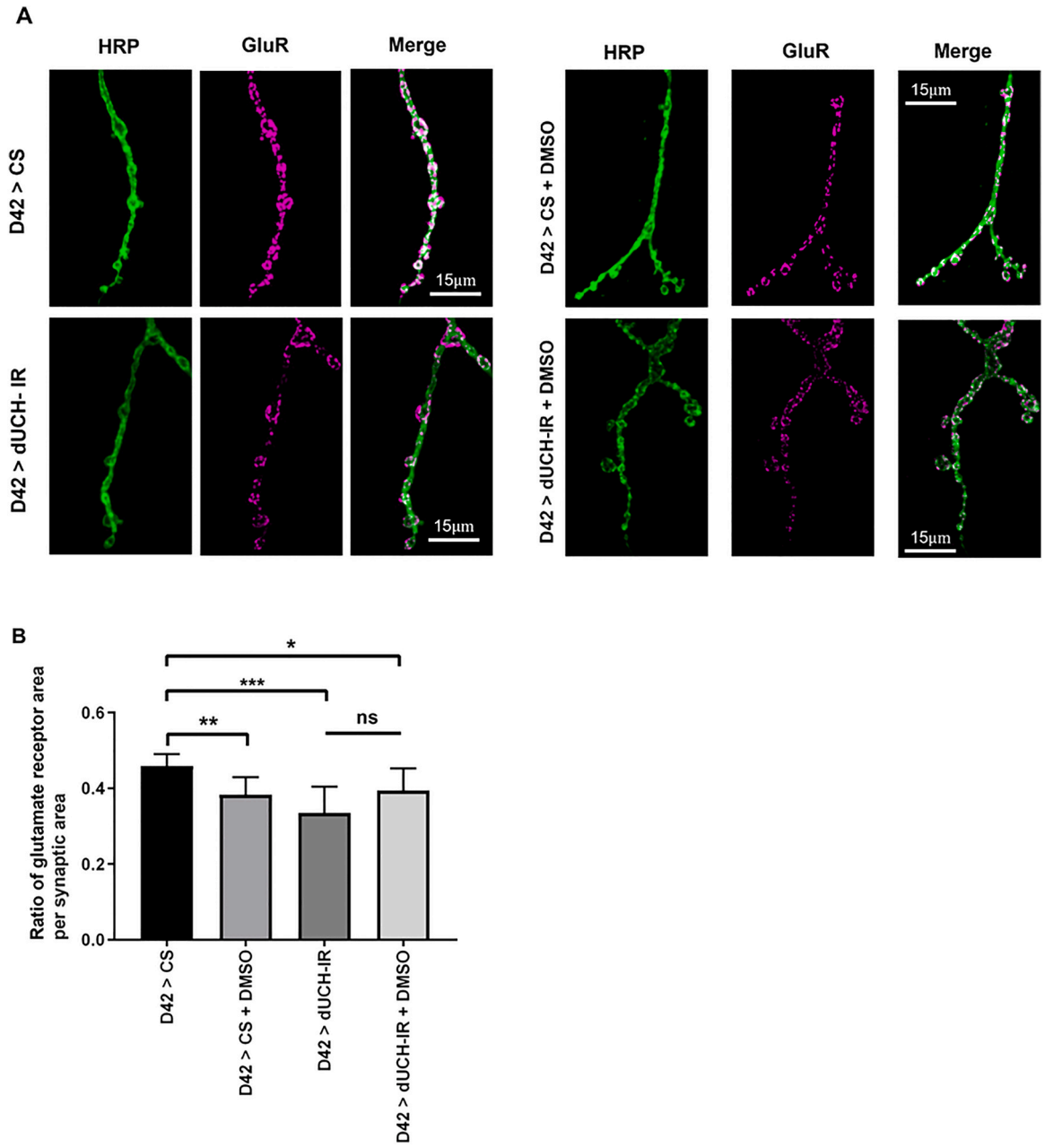

Fig. 3. 0.3 % DMSO affected glutamate receptor density at the NMJ. (A) Double-stained 4th muscle NMJ with anti-HRP IgG (*green*) and anti-GluRIIA IgG (*magenta*). Scale bars: 15 µm. Normal fly: D42 > CS, w/Y; + ; D42-GAL4/+ ; genetic defect fly model: D42 > dUCH-IR, w/Y; + ; D42-GAL4/UAS-dUCH IR. (B) Quantified data for the ratio of glutamate receptor area per synaptic area ($\mu m^2/\mu m^2$). Error bars indicate mean ± SD. Two-tail Student's *t*-test, *p < 0.05, **p < 0.01, ****p < 0.0001, n = 7.

(p = 0.0014) compared to that of the dUCH normal function fly, 0.3 % DMSO helped to reduce the affect (Fig. 4B).

4. Discussion

DMSO is commonly used as a solvent for therapeutic and biological studies. DMSO is frequently used as a solvent for various pharmacological drugs at concentrations of 0.05–1.5 % [30]. Several studies reported that the residual DMSO used for cryoprotection caused adverse neurological responses, including seizures, cerebral infarction, global amnesia, and death after cell-based therapy [16,18,31,44]. Therefore, further study in the toxicity of DMSO is an important issue not only for basic research but also to ensure its safety in humans. Here, our study in *D. melanogaster* model provided evidence which demonstrated that 0.3 % DMSO had effects on the active zone and glutamate receptor (Figs. 1, 3). These results implied that 0.3 % DMSO could induce a reduction in nerve transmission in fly model. Our results were consistent with previous studies that reported the ability of DMSO to decrease or block nerve transmission [13,24,39]. However, difference to the previous studies, the signigicance in this study is the affect of DMSO on neuron cell at low concentration that is in a range of DMSO concentration widely used as pharmacological solvent [32]. These results also contributed a new sight of DMSO affects besides others has been reported such as the effects on inflammation process, cell cycle, differentiation and apoptosis [38]. Notably, this study also revealed the synergistic effect of environmental factors (DMSO 0.3 %) and genetic factors in case of loss function of dUCH as model. The combination caused more serious abnormalities in synapse structure, particularly

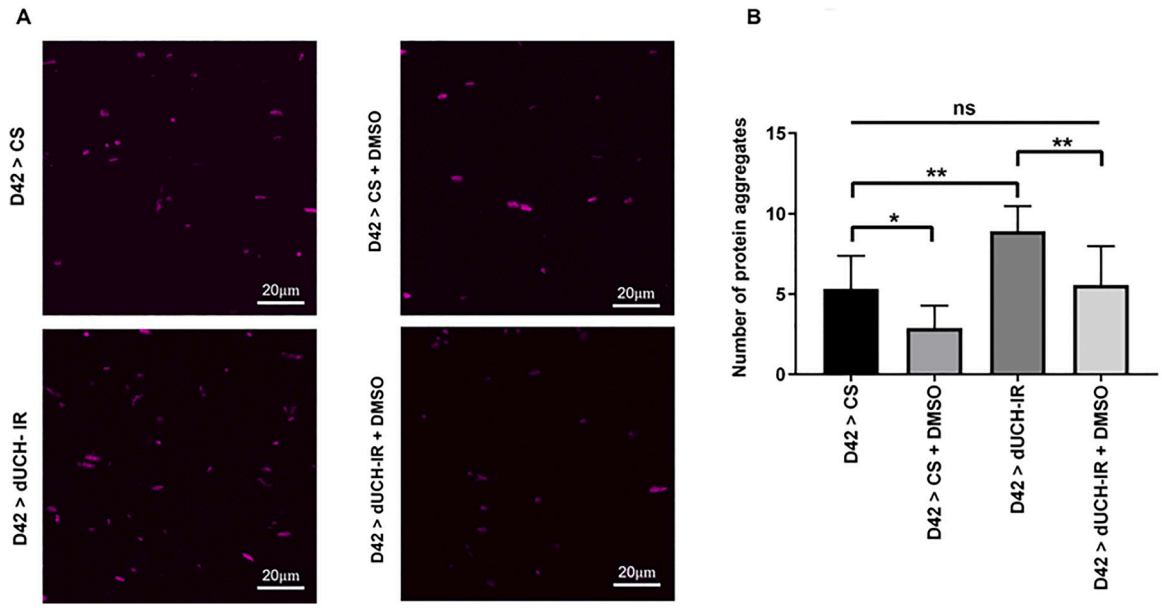

Fig. 4. 0.3 % DMSO decreased the accumulated ubiquitinylated protein in the indirect flight muscle. (A) Indirect flight muscle of *Drosophila* 15 days-old were stained with anti-mono- and poly-ubiquitinylated conjugates (*magenta*). Scale bars: 20 μm. Normal fly: D42 > CS, w/Y; + ; D42-GAL4/+ ; genetic defect fly model: D42 > dUCH-IR, w/Y; + ; D42-GAL4/UAS-dUCH IR. (B) Quantified data of the number of protein aggregates. Error bars indicate mean ± SD. Two-tail Student's *t*-test, *p < 0.05, **p < 0.01, n = 8.

number of boutons on NMJ (Fig. 2 C). While 0.3 % DMSO and dUCH loss function caused the reduction on ratio of glutamate receptor area per synaptic area by 17 % and 27 %, respectively, the affect caused by the combination of 0.3 % DMSO and kdUCH slightly lessened (14 %). Furthermore, 0.3 % DMSO reduced the amount of ubiquitinylated protein aggregates in the indirect flight muscle of both normal and genectic defect fly model. Thereby, it ameliorated the enhancement of ubiquitinylated protein aggregated which caused by loss fucntion of dUCH (Fig. 4). The effect might due to antioxidant activity of DMSO since the well-known antioxidant compound, vitamin C, was also showed the kind of effect as reported in previous study [20]. This observation also suggested that the antioxidant activity of DMSO may disturb the result of experiment which aims to investigate bioactivity of compounds when using DMSO as a solvent.

5. Conclusion

In conclusion, our study indicated that 0.3 % DMSO caused the aberrant morphology of the synaptic structure, active zones and glutamate receptor at NMJs and the decreased the number of ubiquitinylated proteins in the indirect flight muscle of *D. melanogaster* model. The data from the study provided new evidence for the effects of DMSO on the nervous system in *D. melanogaster* model.

Ethics statement

This study was not involved to human or animal subjects.

Funding sources

This research is funded by Vietnam National University, Ho Chi Minh City (VNU-HCM) under grant number 562-2024-18-09.

Authors contributions

Truong Huynh Kim Thoa performed experiments, analyzed the data. Mai Thi Thu Trinh analyzed the data and wrote the manuscript. Dang Thi Phuong Thao analyzed data and edited manuscript. Dang Thi Phuong Thao supervised the study.

CRediT authorship contribution statement

Truong Huynh Kim Thoa: Methodology, Data curation. **Thao Thi Phuong Dang:** Writing – review & editing, Validation, Methodology, Funding acquisition, Formal analysis. **Mai Thi Thu Trinh:** Writing – original draft, Formal analysis, Data curation.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors declare that there is no AI in scientific writing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank all members of Gene Technology and Application group, especially Fly Biomedical Research Group for their great support in this study.

Data Availability

Data will be made available on request.

References

[1] N. Authier, et al., Behavioural assessment of dimethylsulfoxide neurotoxicity in rats, Toxicol. Lett. 132 (2) (2002) 117–121, https://doi.org/10.1016/s0378-4274(02)00052-8.
[2] R. Balázs, et al., Glutamate and glutamate receptors in neurological diseases. Excitatory Amino Acid Transmission in Health and Disease, Oxford University Press, Oxford, UK, 2010, pp. 1–46, https://doi.org/10.1111/j.1532-5415.1995.tb07407.x.

[3] H.J. Bellen, C. Tong, H. Tsuda, 100 years of Drosophila research and its impact on vertebrate neuroscience: a history lesson for the future, Nat. Rev. Neurosci. 11 (7) (2010) 514–522, https://doi.org/10.1038/nrn2839.
[4] Bowman, W., Physiology of the neuromuscular junction, Muscle Relaxants: Physiologic and Pharmacologic Aspects. 2013, Springer. p. 117-127. https://doi.org/10.1007/978-4-431-66896-1_16.
[5] R.D. Broadwell, M. Salcman, R.S. Kaplan, Morphologic effect of dimethyl sulfoxide on the blood-brain barrier, Science 217 (4555) (1982) 164–166, https://doi.org/10.1126/science.7089551.
[6] Anna E. Cartier, et al., Regulation of synaptic structure by ubiquitin C-terminal hydrolase L1, J. Neurosci. 29 (24) (2009) 7857–7868, https://doi.org/10.1523/JNEUROSCI.1817-09.2009.
[7] Anna Castells-Nobau, et al., Two algorithms for high-throughput and multi-parametric quantification of Drosophila neuromuscular junction morphology, J. Vis. Exp. (123) (2017) 55395, https://doi.org/10.3791/55395.
[8] G. Cavaletti, et al., Effect on the peripheral nervous system of systemically administered dimethylsulfoxide in the rat: a neurophysiological and pathological study, Toxicol. Lett. 118 (1-2) (2000) 103–107, https://doi.org/10.1016/S0378-4274(00)00269-1.
[9] J.X. Comella, J. Molgo, L. Faille, Sprouting of mammalian motor nerve terminals induced by in vivo injection of botulinum type-D toxin and the functional recovery of paralysed neuromuscular junctions, Neurosci. Lett. 153 (1) (1993) 61–64, https://doi.org/10.1016/0304-3940(93)90077-X.
[10] Cvetković, V., et al. (2013). Toxicity of commonly used solvent dimethyl sulfoxide against Drosophila melanogaster larvae: determination of LC50, LOEC and NOEC values. Proceedings of the 11th Symposium on the Flora of Southeastern Serbia and Neighbouring Regions, Vlasina, Serbia. pp: 13-16.
[11] L.E. DeForge, et al., Oxygen radical scavengers selectively inhibit interleukin 8 production in human whole blood, J. Clin. Investig. 90 (5) (1992) 2123–2129, https://doi.org/10.1172/JCI116097.
[12] A.M. Di Giorgio, et al., Dimethyl sulfoxide provides neuroprotection in a traumatic brain injury model, Restor. Neurol. Neurosci. 26 (6) (2008) 501–507, https://doi.org/10.3389/fphar.2022.998179.
[13] J.A. Donoso, J.-P. Illanes, F. Samson, Dimethylsulfoxide action on fast axoplasmic transport and ultrastructure of vagal axons, Brain Res. 120 (2) (1977) 287–301, https://doi.org/10.1016/0006-8993(77)90907-6.
[14] J.J. Fernandes, H. Keshishian, Nerve-muscle interactions during flight muscle development in Drosophila, Development 125 (9) (1998) 1769–1779, https://doi.org/10.1242/dev.125.9.1769.
[15] J.L. Hanslick, et al., Dimethyl sulfoxide (DMSO) produces widespread apoptosis in the developing central nervous system, Neurobiol. Dis. 34 (1) (2009) 1–10, https://doi.org/10.1016/j.nbd.2008.11.006.
[16] M. Higman, et al., Reversible leukoencephalopathy associated with re-infusion of DMSO preserved stem cells, Bone Marrow Transplant. 26 (7) (2000) 797–800, https://doi.org/10.1038/sj.bmt.1702589.
[17] N. Hirsch, Neuromuscular junction in health and disease, Br. J. Anaesth. 99 (1) (2007) 132–138, https://doi.org/10.1093/bja/aem144.
[18] R. Hoyt, J. Szer, A. Grigg, Neurological events associated with the infusion of cryopreserved bone marrow and/or peripheral blood progenitor cells, Bone Marrow Transplant. 25 (12) (2000) 1285–1287, https://doi.org/10.1038/sj.bmt.1702443.
[19] L.C. Hunt, F. Demontis, Whole-mount immunostaining of Drosophila skeletal muscle, Nat. Protoc. 8 (12) (2013) 2496–2501, https://doi.org/10.1038/nprot.2013.156.
[20] T.K.T. Huynh, et al., Crucial roles of ubiquitin carboxy-terminal hydrolase L1 in motor neuronal health by drosophila model, Antioxid. Redox Signal. 37 (4-6) (2022) 257–273, https://doi.org/10.1089/ars.2021.0057.
[21] S. Jawkar, U. Nongthomba, Indirect flight muscles in Drosophila melanogaster as a tractable model to study muscle development and disease, Int. J. Dev. Biol. 64 (1-2-3) (2020) 167–173, https://doi.org/10.1387/ijdb.190333un.
[22] B.H. Jennings, Drosophila–a versatile model in biology & medicine, Mater. Today 14 (5) (2011) 190–195, https://doi.org/10.1016/S1369-7021(11)70113-4.
[23] T. Iwasaki, et al., A case of pulmonary amyloidosis associated with multiple myeloma successfully treated with dimethyl sulfoxide, Acta Haematol. 91 (2) (1994) 91–94, https://doi.org/10.1159/000204262.
[24] J. Larsen, K. Gasser, R. Hahin, An analysis of dimethylsulfoxide-induced action potential block: a comparative study of DMSO and other aliphatic water soluble solutes, Toxicol. Appl. Pharmacol. 140 (2) (1996) 296–314, https://doi.org/10.1006/taap.1996.0225.
[25] J. Liu, et al., Involvement of mitochondrial permeability transition and caspase-9 activation in dimethyl sulfoxide-induced apoptosis of EL-4 lymphoma cells, Int. Immunopharmacol. 1 (1) (2001) 63–74, https://doi.org/10.1016/S1567-5769(00)00016-3.
[26] K.A. McCammon, et al., Intravesical dimethyl sulfoxide for primary amyloidosis of the bladder, Urology 52 (6) (1998) 1136–1138, https://doi.org/10.1016/S0090-4295(98)00347-1.
[27] K.P. Menon, R.A. Carrillo, K. Zinn, Development and plasticity of the Drosophila larval neuromuscular junction, Wiley Interdiscip. Rev.: Dev. Biol. 2 (5) (2013) 647–670, https://doi.org/10.1002/wdev.108.
[28] J.J. Modrzyński, J.H. Christensen, K.K. Brandt, Evaluation of dimethyl sulfoxide (DMSO) as a co-solvent for toxicity testing of hydrophobic organic compounds, Ecotoxicology 28 (9) (2019) 1136–1141, https://doi.org/10.1007/s10646-019-02107-0.
[29] P. Morassi, et al., Treatment of amyloidosis with dimethyl sulfoxide (DMSO), Minerva Med. 80 (1) (1989) 65–70, https://pubmed.ncbi.nlm.nih.gov/2915815/.
[30] M. Moskot, et al., The role of dimethyl sulfoxide (DMSO) in gene expression modulation and glycosaminoglycan metabolism in lysosomal storage disorders on an example of mucopolysaccharidosis, Int. J. Mol. Sci. 20 (2) (2019) 304, https://doi.org/10.3390/ijms20020304.
[31] L.P. Mueller, et al., Neurotoxicity upon infusion of dimethylsulfoxide-cryopreserved peripheral blood stem cells in patients with and without pre-existing cerebral disease, Eur. J. Haematol. 78 (6) (2007) 527–531, https://doi.org/10.1111/j.1600-0609.2007.00851.x.
[32] Aamir Nazir, et al., Evaluation of the no observed adverse effect level of solvent dimethyl sulfoxide in *Drosophila melanogaster*, Toxicol. Mech. Methods 13 (2) (2003) 147–152, https://doi.org/10.1080/15376510309846.
[33] Dang Ngoc Anh Suong, et al., Ubiquitin carboxyl hydrolase L1 significance for human diseases, Protein Pept. Lett. 21 (7) (2014) 624–630, https://doi.org/10.2174/0929866521666140403125959.
[34] S. Oka, et al., A correlation of reactive oxygen species accumulation by depletion of superoxide dismutases with age-dependent impairment in the nervous system and muscles of Drosophila adults, Biogerontology 16 (2015) 485–501, https://doi.org/10.1007/s10522-015-9570-3.
[35] J. Parkin, C. Shea, G.R. Sant, Intravesical dimethyl sulfoxide (DMSO) for interstitial cystitis–a practical approach, Urology 49 (5) (1997) 105–107.
[36] W. Regelson, S. Harkins, Amyloid Is Not a Tombstone"—a summation: the primary role for cerebrovascular and CSF dynamics as factors in Alzheimer's disease (AD): DMSO, fluorocarbon oxygen carriers, thyroid hormonal, and other suggested therapeutic measures, Ann. N. Y. Acad. Sci. 826 (1) (1997) 348–374, https://doi.org/10.1111/j.1749-6632.1997.tb48485.x.
[37] J.B.T. Rocha, Drosophila melanogaster as a promising model organism in toxicological studies, Arch. Basic Appl. Med. 1 (1) (2013) 33–38, https://doi.org/10.1016/j.pestbp.2019.09.006.
[38] N.C. Santos, et al., Multidisciplinary utilization of dimethyl sulfoxide: pharmacological, cellular, and molecular aspects, Biochem. Pharmacol. 65 (7) (2003) 1035–1041, https://doi.org/10.1016/S0006-2952(03)00002-9.
[39] K. Somei, et al., Dimethylsulfoxide potentiates the suppressive effects of lidocaine on synaptic transmission in bullfrog sympathetic ganglion, Methods Find. Exp. Clin. Pharmacol. 17 (9) (1995) 571–576. ⟨https://europepmc.org/article/med/8786669⟩.
[40] Caroline M. Tanner, et al., Rotenone, paraquat, and Parkinson's disease, Environ. Health Perspect. 119 (6) (2011) 866–872, https://doi.org/10.1289/ehp.1002839.
[41] Thao, D.T.P. (2018). Drosophila Model in the Study Role of UCH-L1. In Drosophila melanogaster-Model for Recent Advances in Genetics and Therapeutics. IntechOpen. https://doi.org/10.5772/INTECHOPEN.73578.
[42] E. Toraman, Biochemical and molecular evaluation of oxidative stress and mitochondrial damage in fruit fly exposed to carmoisine, Mol. Biol. Rep. 51 (1) (2024) 685, https://doi.org/10.1007/s11033-024-09616-0.
[43] Sagar Verma, et al., Neuromuscular junction dysfunction in amyotrophic lateral sclerosis, Mol. Neurobiol. (2022) 1–26, https://doi.org/10.1007/s12035-021-02658-6.
[44] P. Windrum, T. Morris, Severe neurotoxicity because of dimethyl sulphoxide following peripheral blood stem cell transplantation, 315-315, Bone Marrow Transplant. 31 (4) (2003), https://doi.org/10.1038/sj.bmt.1703848.
[45] C. Yuan, et al., Dimethyl sulfoxide damages mitochondrial integrity and membrane potential in cultured astrocytes, PloS One 9 (9) (2014) e107447, https://doi.org/10.1371/journal.pone.0107447.