

Protopanaxadiol-type ginsenosides ameliorate PTSD-like memory impairment and neuroinflammation in rats via suppression of TLR4/NF- κ B signaling

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Abstract. Post-traumatic stress disorder (PTSD) is a serious psychiatric condition that develops after exposure to intense or traumatic stress. Ginsenosides, the primary bioactive compounds of ginseng, have been reported to enhance cognitive performance. Protopanaxadiol (PD) and protopanaxatriol (PT) are two major classes of triterpenoid saponins. In the present study, a PTSD-like rat model was established using the single prolonged stress (SPS) paradigm, and the effects of PD and PT on memory dysfunction were evaluated. After SPS exposure, male rats received daily intraperitoneal administration of PD (10 or 20 mg/kg) or PT (10 and 20 mg/kg) for 14 days. PD treatment significantly improved both spatial and cognitive memory compared with PT treatment. PD also suppressed the elevation of pro-inflammatory cytokines in the prefrontal cortex and hippocampus, and mitigated the reduction in brain-derived neurotrophic factor mRNA expression in the hippocampus. In addition, PD attenuated the upregulation of Toll-like receptor 4 (TLR4) and nuclear factor (NF)- κ B protein expression in the hippocampus. By contrast, PT treatment did not produce meaningful changes in memory performance or inflammatory markers. These findings suggest that the beneficial effects of PD are associated with anti-inflammatory activity that may involve modulation of the TLR4/NF- κ B signaling pathway.

Introduction

Post-traumatic stress disorder (PTSD) is a serious psychiatric condition that develops after exposure to traumatic or highly stressful events, including violent crime, traffic accidents,

natural disasters, terrorist incidents, occupational stress, and sexual violence (1). PTSD affects approximately 7% of the population at some point during life (2). In addition, the COVID-19 pandemic was associated with a marked increase in PTSD symptoms among healthcare workers and patients worldwide.

PTSD is associated with a range of pathological symptoms, including anxiety, depression, intrusive memories, and impairments in learning and memory (1). Unlike general chronic stress, PTSD is characterized by persistent fear responses and difficulty extinguishing traumatic memories, often accompanied by alterations in cognitive function and brain activity (3).

At the biological level, PTSD is closely linked to neuroinflammatory processes, including dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and increased production of pro-inflammatory cytokines such as interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α), in the brain (4). Neuroinflammation in the medial prefrontal cortex (PFC) and hippocampus is particularly relevant because these regions regulate cognitive and memory functions and contribute to other neuropsychiatric symptoms (5).

Accordingly, reducing neuroinflammation may help mitigate behavioral abnormalities, including PTSD-related memory impairment, and may contribute to neuronal protection (5). Among the molecular mechanisms involved, Toll-like receptor 4 (TLR4) is one of the most extensively studied members of the TLR family. Activation of TLR4 triggers downstream signaling cascades that promote cytokine release and activate the nuclear factor (NF)- κ B pathway (6). These pathways are critically involved in inflammation, apoptosis, and cognitive dysfunction, including deficits in learning and memory.

Previous studies have shown that PTSD is associated with synaptic loss and altered synaptic plasticity, both of which are essential for memory formation and storage (7). PTSD has also been reported to affect the expression of brain-derived neurotrophic factor (BDNF), a key regulator of neuronal growth and survival, and its receptor, tropomyosin receptor kinase B (TrkB) (8).

The BDNF-TrkB signaling pathway interacts with intracellular cascades, including activation of the NF- κ B pathway, which plays a central role in regulating neuroinflammatory

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responses such as pro-inflammatory cytokine production. These processes are closely linked to neuronal survival and synaptic plasticity (8). Therefore, modulation of inflammatory cytokines and NF- κ B activity, together with regulation of the BDNF-TrkB signaling pathway, may improve memory function by influencing synaptic plasticity (9).

Although commonly prescribed antidepressants for PTSD can provide symptomatic relief, their therapeutic effects are often temporary, and their clinical use is limited by significant adverse effects. These limitations indicate the need for additional therapeutic strategies (10).

Ginseng (*Panax ginseng* C.A. Meyer) has been used for centuries as a traditional medicinal herb and is widely recognized for its anti-fatigue, antioxidant, anti-allergic, anti-tumor, and anti-inflammatory properties in chronic diseases (11). More recently, ginseng has attracted attention for its potential benefits in neurodegenerative disorders, psychiatric conditions, and stress-related memory impairment, with evidence suggesting improvements in learning and cognitive performance (12).

The primary bioactive constituents of ginseng are ginsenosides, a class of glycosides composed of an aglycone backbone—either protopanaxadiol (PD) or protopanaxatriol (PT). These compounds exhibit diverse pharmacological activities (13,14). Previous studies have reported that ginsenoside Rb1, a representative PD-type saponin, enhances learning and memory in amyloid beta (A β)-induced Alzheimer's disease (AD) mouse models, likely through its anti-inflammatory effects (15). Similarly, ginsenoside Rg3, another PD-type compound, has been shown to attenuate cognitive deficits by suppressing inflammatory mediator expression in lipopolysaccharide (LPS)-induced models of memory impairment (16). In contrast, ginsenoside Rg1, a PT-type saponin, has demonstrated efficacy in improving cognitive function under postmenopausal conditions and provides neuroprotective effects in the hippocampus in ovariectomized and D-galactose-treated mouse models of AD (17). Collectively, these findings suggest that both PD- and PT-type ginsenosides may have therapeutic potential for alleviating neuroinflammation and memory deficits associated with PTSD.

In this study, we compared the effects of PD and PT on PTSD-induced memory impairment using a single prolonged stress (SPS) rat model. We further examined whether these compounds exert anti-inflammatory effects through modulation of the TLR4/NF- κ B signaling pathway, which plays a critical role in cognitive function.

Materials and methods

Animals and PD or PT-type saponins administration. Male Sprague-Dawley rats aged 7–8 weeks and weighing 200–220 g were obtained from Samtaco Animal Co. (Seoul, Korea). The rats were housed in an animal room under a 12 h light/dark cycle and were provided sterile drinking water and food *ad libitum*. Animal experiments were conducted in accordance with the Code of Ethics and guidelines for the management of laboratory animals by the Animal Care and Use Committee of Kyung Hee University (Seoul, South Korea), which also approved the experimental protocol (approval no. KHSASP-21-268). Before behavioral testing, the rats were allowed to acclimatize for 1

week and were handled daily by the experimenter for at least 1 week to minimize stress-related effects during the experiment. The rats were randomly divided into 6 groups, with 6 to 7 rats per group.

Panaxadiol (10 and 20 mg/kg, Merck, Darmstadt, Germany) and panaxatriol (10 and 20 mg/kg, Merck), both extracted from red ginseng and paroxetine (10 mg/kg, PAX; Sigma-Aldrich Chemical Co., St. Louis, MO, USA, positive control) were intraperitoneally injected once daily for 14 days. Based on previous research findings, intraperitoneal administration was selected to achieve rapid systemic absorption and ensure safe drug delivery (8). These drugs were dissolved in 0.9% physiological saline immediately before use. The dosages of PD and PT were determined based on previous studies (13,14). Forty-nine rats were randomly divided into the following seven groups ($n=7$ rats/group). The detailed experimental schedule is presented in Fig. 1. All behavioral measurements were performed by an independent investigator who was blinded to the experimental conditions.

Single prolonged stress. To establish a PTSD-like animal model, rats were subjected to restraint stress for 2 h and forced swimming for 20 min. The rats were then allowed to rest for 15 min and were exposed to isoflurane (2–3%) until loss of consciousness, as confirmed by absence of righting reflex and lack of response to toe and tail pinch (<5 min). Isoflurane was used only for induction at 2–3% until loss of consciousness and was not maintained thereafter. This procedure followed the SPS method (8). After SPS exposure, the rats were left undisturbed for 7 days to allow sensitization.

Contextual fear conditioning and extinction. The effects of PD and PT on learned fear were assessed using the conditioned fear test. Conditioned fear is a learned response in which animals associate sensory cues, such as environmental context or sound, with a life-threatening or painful stimulus (18). On day 1, rats were placed in a computerized fear-conditioning chamber using the Gemini Avoidance System (SD Instruments, San Diego, CA, USA). A conditioned stimulus (CS; 30 sec, 85dB) was paired with an electric shock as the unconditioned stimulus (US; 0.5 mA, 3 sec) to induce fear memory. The white noise (85 dB) and chamber light were turned on 28 sec before shock, delivery and were turned off when the shock ended. Thus, the CS lasted 30 sec, and the electric shock was delivered during the final 2 sec. This session was repeated 4 times, with an intertrial interval of 2 min. On day 2, rats underwent extinction training consisting of 12 CS presentations without shock. On day 3, rats received 3 CS-only presentations to test extinction recall (in the same context). Fear responses were observed for 5 min. Freezing was defined as the absence of movement except respiration during video observation, and the total freezing time was measured and compared among experimental groups.

Object recognition task. To assess object recognition memory, the object recognition test (ORT) was performed (8). Rats were allowed to explore objects in a black acrylic box (40x40x25 cm). Exploratory behavior was measured as the basis for object discrimination. Two similar wooden toys (familiar objects, A1 and A2) and one novel wooden toy (B) with a completely

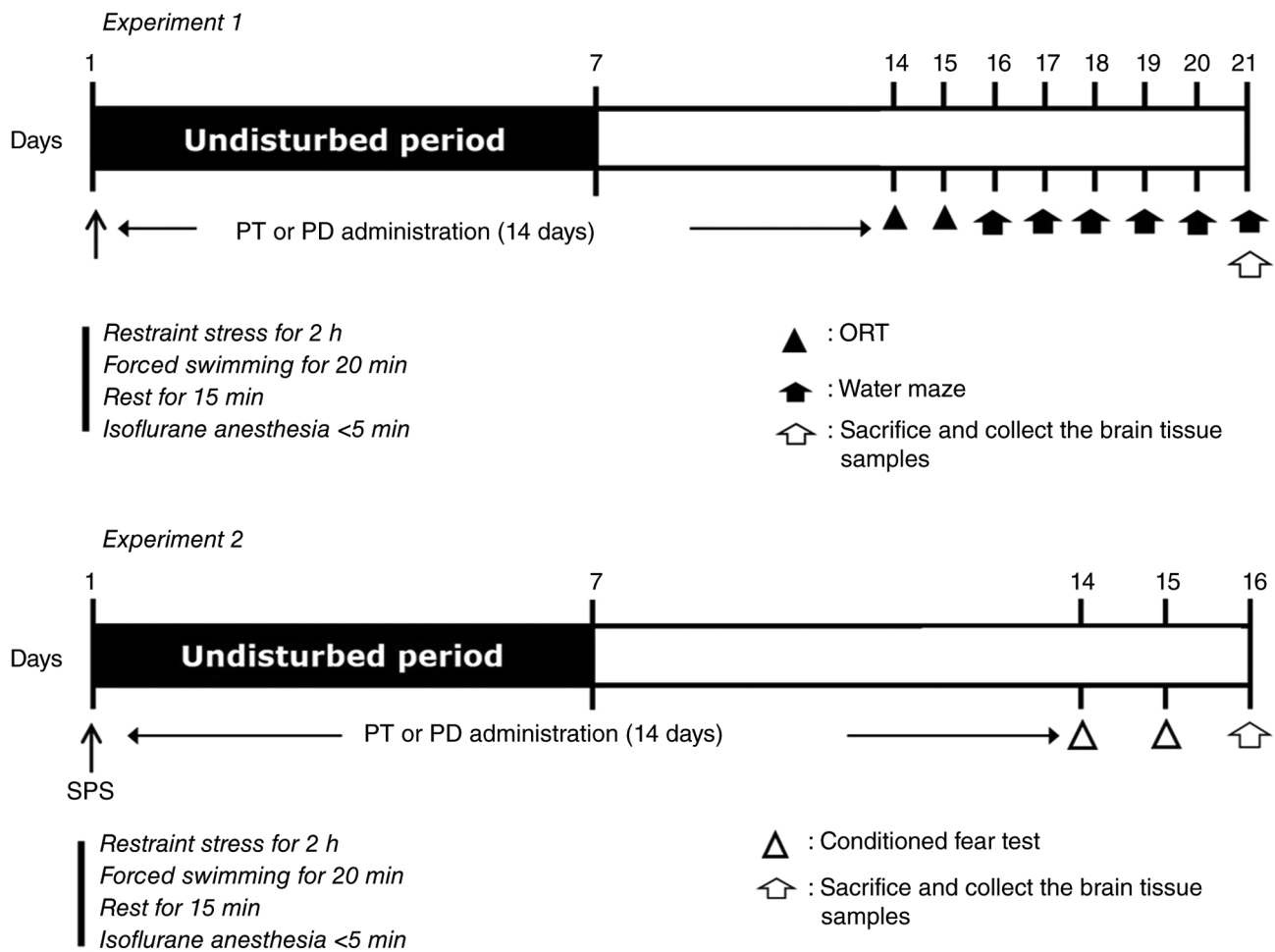


Figure 1. Experimental protocols for SPS-induced memory impaired behaviors and ginsenoside PD or PT treatment in rats. Different groups of rats (n=7/group) were used for each experimental condition. ORT, object recognition test; PD, protopanaxadiol; PT, protopanaxatriol; SPS, single prolonged stress.

different color and shape were used. The objects were large enough that the rats could not easily move them. On the first day, rats were acclimatized to the experimental environment for 10 min. After 24 h, during the training phase, rats were placed in the box with 2 similar toys (A1 and A2) and allowed to explore for 5 min. After 2 h, during the test phase, rats were exposed to one novel wooden toy (B) and one familiar object (A1) for 5 min. Sniffing time directed toward novel or familiar objects was measured. Exploration was defined as active sniffing of the object, touching the object with the forepaw, or directing the snout toward the object.

Morris water maze test. The Morris water maze (MWM) is widely used to evaluate spatial learning and memory in rats (8). Acquisition and retention were assessed using the MWM test. The apparatus consisted of a circular plastic tank (diameter, 200 cm; height, 35 cm) filled with water a depth of approximately 22 cm. The tank was divided into quadrants, and an escape platform (height, 20 cm; diameter, 15 cm) was placed in a randomly selected quadrant. The platform was positioned 1.0 cm below the water surface, and the water was made opaque with paint to conceal the platform. Rats were placed into the tank from the central wall of a quadrant that did not contain the platform. Escape latency was measured from placement in the tank until the rat found the platform.

The acquisition test was conducted 3 times per day for 5 days. When a rat reached the platform, it remained there for 10 sec before the next trial. If the rat did not locate the platform within 180 sec, the researcher guided it to the platform and allowed it to remain there for 10 sec to learn the platform location. Acquisition trials were repeated at 60 min intervals. On day 6, the escape platform was removed, and spatial memory retention was assessed during a 60 sec probe trial in the quadrant where the platform had been located. All experimental procedures were video-recorded using SMART software (version 2.5; Panlab, Cornella, Spain) installed on a ceiling-mounted system. Escape latency was analyzed from the recorded data using the image analysis system.

Enzyme-linked immunosorbent assay analysis of inflammatory mediators and NF- κ B protein levels. After behavioral measurements, rats (n=4) were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.) according to the approved IACUC protocol. Adequate anesthesia was confirmed by the absence of the forelimb withdrawal reflex. Under deep anesthesia, euthanasia was performed by cervical dislocation, followed by thoracotomy, cardiac blood collection, and rapid removal of the whole brain for subsequent analyses. Frontal cortex was firstly taken out. After overlying temporal cortex was unveiled, the hippocampus was exposed and collected for use. Blood was

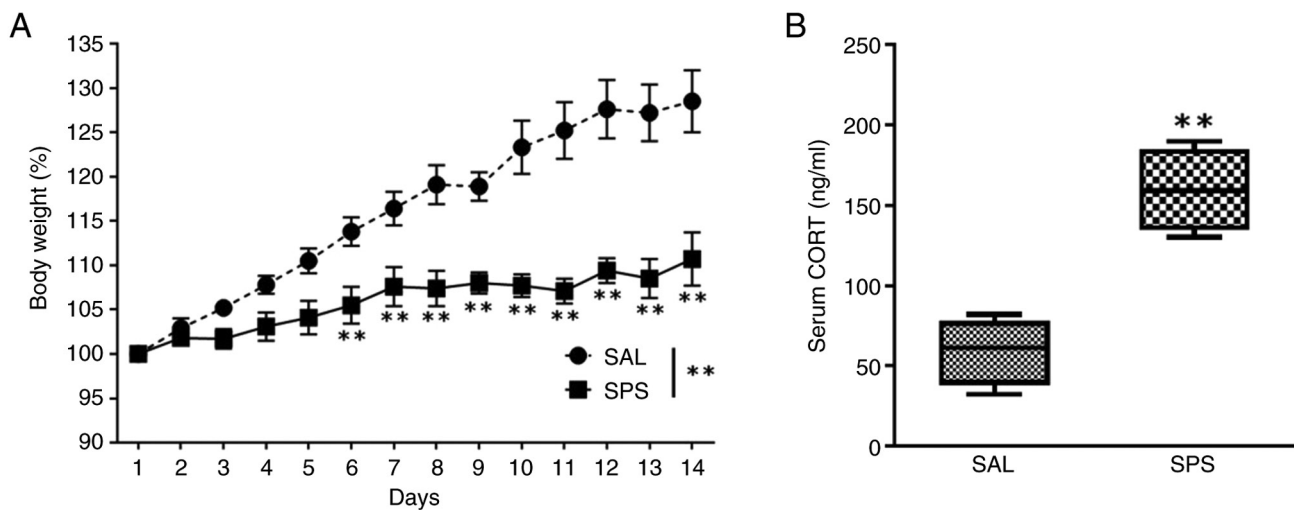


Figure 2. Results of body weight and serum corticosterone analyses in rats subjected to 14 days of a SPS. (A) Body weights and (B) plasma corticosterone levels were analyzed in SPS-exposed and SAL-treated rats. Data are shown as the mean $\pm$ standard error of the mean. ** $P < 0.01$ vs. SAL group. SAL, saline; SPS, single prolonged stress.

sampled from the heart at the time of euthanasia. The brain of rats was obtained, and pro-inflammatory cytokine levels in the PFC and hippocampus were quantified using enzyme-linked immunosorbent assay (ELISA). The concentrations of TNF- α and IL-1 β (Abcam, Cambridge, MA, USA), as well as interleukin-6 (IL-6; Cell Signaling Technology, Danvers, MA, USA), were determined in both brain regions according to previously established protocols. In addition, hippocampal levels of TLR4 (NOVUS Biologicals, Chesterfield, MO, USA) and NF- κ B (MyBioSource, San Diego, CA, USA) were analyzed using a sandwich ELISA method, following standard procedures described in earlier studies.

Total RNA isolation and RT-PCR analysis. After the rats were euthanized under anesthesia, the hippocampus ($n=3$ /group) was isolated, added to TRIzol[®] reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and vortexed to recover total RNA. After RNA was reverse transcribed into cDNA, PCR was performed using primers specific to each target, and PCR reagents were selected according to previous reports (8). PCR was performed at 57°C for 27 cycles for BDNF and at 58°C for 28 cycles for TrkB using a thermal cycler (MJ Research, Watertown, MA, USA). PCR products were confirmed by electrophoresis on 1.5% agarose gels. The following primer sequences were used for PCR: GAPDH (228 bp), (forward) 5'-CTCCTGAAGTTCAGGCAGTTTA-3' and (reverse) 5'-GTAACCAAGTCATCAGCAAATGT-3'; BDNF (227 bp): 5'-GCTGAGCGTGTGTGACAGTA-3' (sense) and 5'-ACTGGGTAGTTCGGCACTGG-3' (antisense); TrkB (283 bp): 5'-GAGTTTCTGCCCTGCTCTG-3' (sense) and 5'-GGACTCTTTGGGTGCAGAA-3' (antisense).

Statistical analysis. All data was expressed as the mean $\pm$ SEM. Statistical differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test in SPSS software (version 23.0; SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Effects of body weight and serum CORT levels following single prolonged stress. Analysis of body weight over 14 days showed that the saline-treated normal (SAL) group gained body weight over time (Fig. 2A). In contrast, the SPS group showed reduced body weight gain compared to the SAL group ($t=0.514$, $P < 0.001$). Although these body weight changes are not directly related to SPS-induced memory impairment, body weight is an important indicator of physiological status in rats. Therefore, the reduced body weight gain in SPS-exposed rats suggests that the animals underwent physiological changes in response to traumatic stress.

After body weight measurement, serum CORT levels were assessed in SPS-exposed rats (Fig. 2B). Serum CORT levels were significantly higher in the SPS group than in the SAL group ($P < 0.01$). These results indicate that SPS induced a stress response accompanied by changes in body weight.

Effects of PD and PT-type saponins on SPS-induced freezing response. Following administration of PT and PD in the SPS-induced animal model of memory impairment, fear responses were assessed. Freezing response during extinction trials and short-term extinction recall was used to determine whether SPS induced a conditioned fear response (Fig. 3A). During fear conditioning, no difference in freezing response was observed between the SAL and SPS groups ($t=0.237$, $P < 0.01$). However, freezing responses differed significantly between the 2 groups during extinction learning ($t=0.284$, $P < 0.05$) and extinction recall ($t=0.845$, $P < 0.05$). The PD20 group showed a significant decrease in freezing response during extinction recall compared with the SPS group ($P < 0.05$; Fig. 3B), whereas the PT group did not affect freezing response during extinction recall. These results indicate that SPS induced a stress-related conditioned fear response in rats and that PD administration may inhibit SPS-induced conditioned fear memory formation or promote fear-memory extinction.

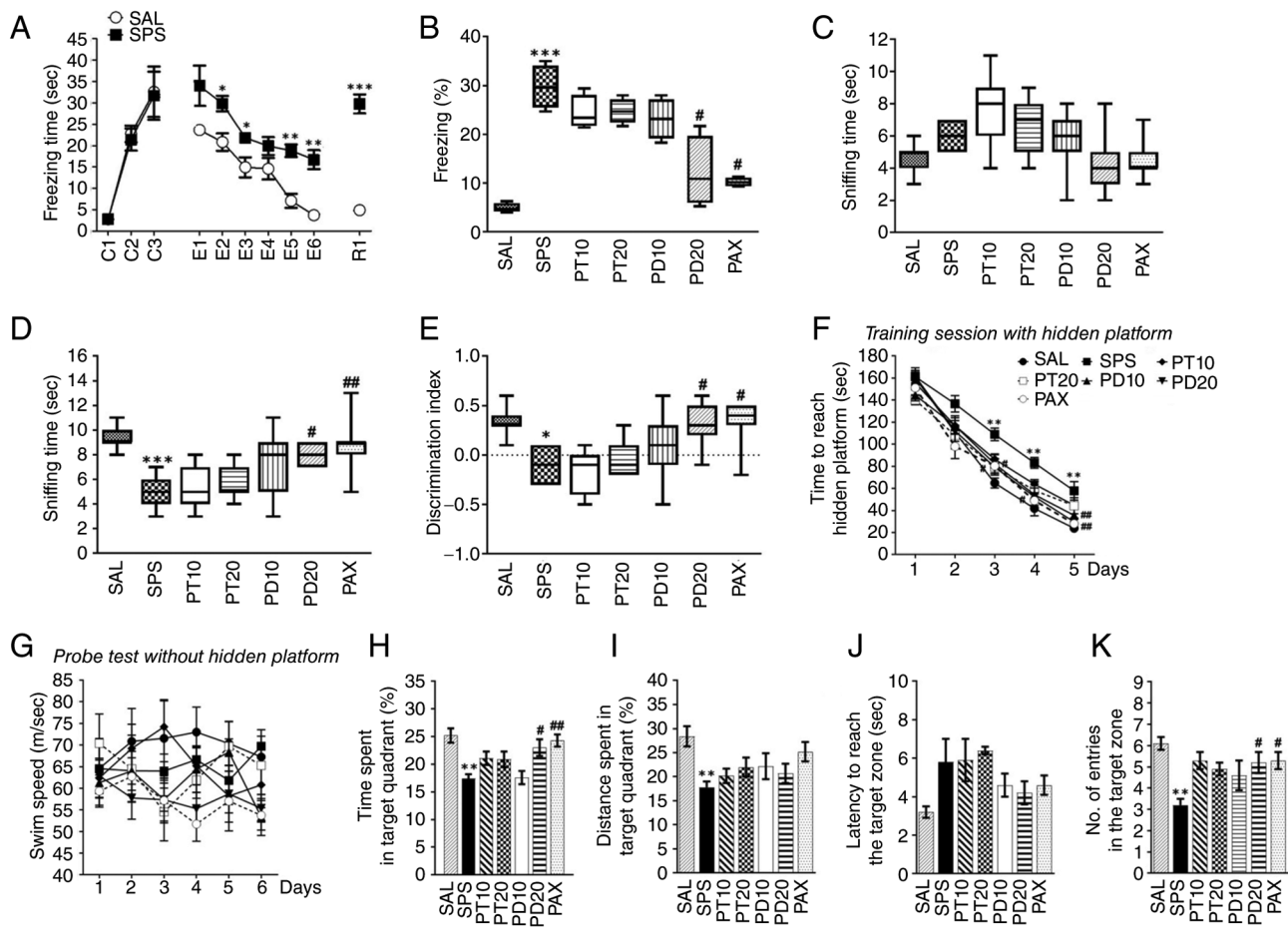


Figure 3. Effect of PD and PT-type saponins on SPS-induced memory impairment. Comparison of ginsenoside PD or PT effects on (A) freezing behavior during conditioning, extinction, and short-term recall and the (B) percentages of time spent freezing during short-term recall in contextual fear conditioning. Comparison of ginsenoside PD or PT effects on (C) exploration time for two familiar objects, (D) exploration time for familiar and novel objects, and the (E) ability to discriminate between familiar and new objects in the novel object recognition test. Comparison of ginsenoside PD or PT effects on (F) time to escape (latency) from the water onto a submerged platform during acquisition trials, (G) swimming speed, (H) percentage of time spent in the target quadrant, (I) percentage of distance traveled in the target quadrant, (J) path efficiency to reach the target zone and (K) number of entries into the target zone in the Morris water maze. *P<0.05, **P<0.01, ***P<0.001 vs. SAL group; #P<0.05, ##P<0.01 vs. SPS group. PD, protopanaxadiol; PT, protopanaxatriol; SAL, saline; SPS, single prolonged stress; PAX, paroxetine; C1-3, fear conditioning sessions; E1-6, extinction training sessions; R1, extinction recall session.

Effects of PD and PT-type saponins on SPS-induced impairments in cognitive and spatial memory. The effects of PT and PD on cognitive function were evaluated in the SPS-induced animal model of memory impairment. In the ORT, no significant differences were observed among groups in the exploration time of familiar objects (Fig. 3C). However, the SPS group showed a marked reduction in sniffing time for novel objects compared with the SAL group (P<0.001; Fig. 3D). In contrast, the PD20-treated group showed a significant increase in novel-object exploration time compared with the SPS group (P<0.05), whereas no significant change was observed in the PT-treated group. The discrimination index was also significantly higher in the PD20 group than in the SPS group (P<0.05; Fig. 3E). These findings indicate that SPS markedly impaired object recognition memory in rats, whereas PD administration ameliorated SPS-induced cognitive deficits.

The effects of PT and PD on spatial learning and memory were evaluated using the SPS-induced model of spatial cognitive impairment. During the 5-day acquisition phase of the MWM test, the SPS group required significantly more time to locate the hidden platform than the SAL group (P<0.01

on days 3-5; Fig. 3F), indicating impaired spatial learning. In contrast, the PD20-treated group showed a significantly shorter escape latency than the SPS group (P<0.05 on days 3 and 4 and P<0.01 on day 5), whereas the PT-treated group did not show a meaningful reduction in escape latency. No significant differences in mean swimming speed was observed among groups (Fig. 3G), suggesting that the observed differences in escape latency were not attributable to altered motor function.

In the probe test assessing spatial memory, the SPS group showed significant reductions in time spent in the target quadrant, distance traveled within the target area, and number of entries into the target zone compared with the SAL group (P<0.01 for all comparisons; Fig. 3H-K). Conversely, the PD20 group showed significant increases in time spent in the target area and number of target entries compared with the SPS group (P<0.05 for each measure). These findings indicate that PD administration improved SPS-induced deficits in spatial learning and memory.

Effects of PD and PT-type saponins on pro-inflammatory cytokines levels in the PFC and hippocampus following SPS. After PT or PD administration in the SPS-induced model

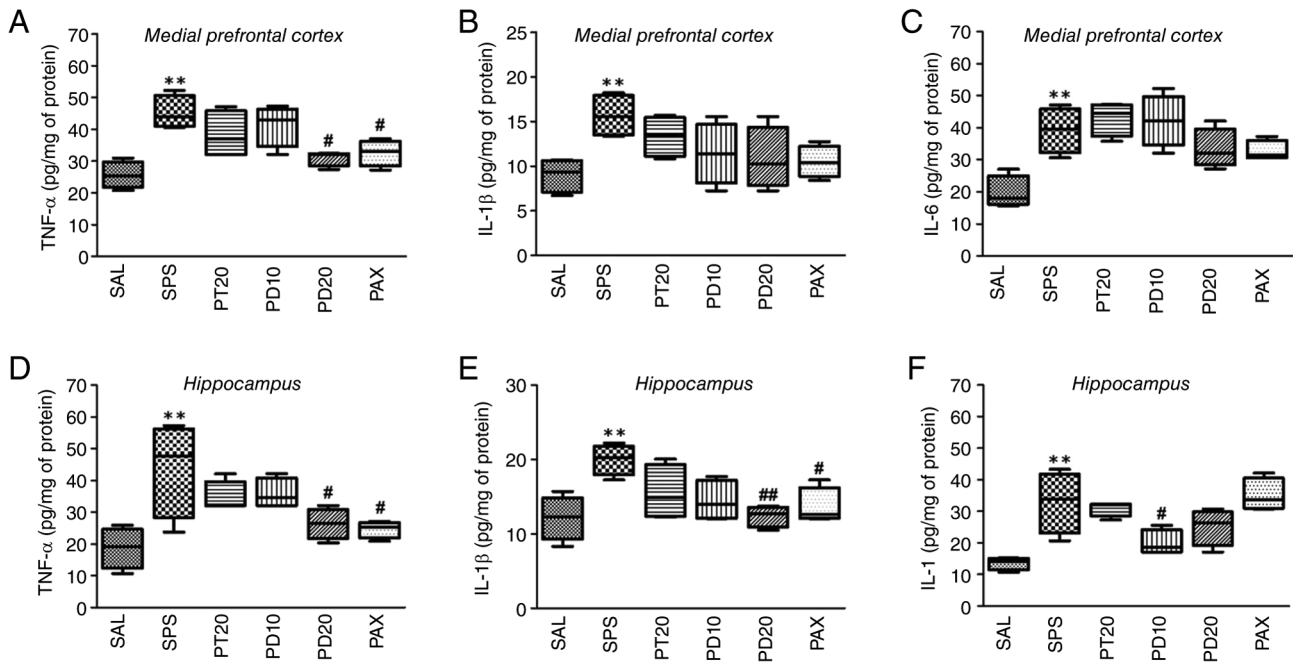


Figure 4. Effect of PD and PT-type saponins on SPS-induced neuroinflammation. Comparison of ginsenoside PD or PT effects on (A) TNF- α , (B) IL-1 β and (C) IL-6 levels in the medial prefrontal cortex, and (D) TNF- α , (E) IL-1 β and (F) IL-6 levels in the hippocampus of rats exposed to SPS as measured by enzyme-linked immunosorbent assay. ** $P < 0.01$ vs. SAL group; # $P < 0.05$, ## $P < 0.01$ vs. SPS group. IL, interleukin; PD, protopanaxadiol; PT, protopanaxatriol; SAL, saline; SPS, single prolonged stress; PAX, paroxetine; TNF- α , tumor necrosis factor- α .

of memory impairment, inflammatory cytokine levels were measured in the PFC and hippocampus. The SPS group showed significantly elevated concentrations of pro-inflammatory cytokines, including TNF- α , IL-1 β , and IL-6, in both brain regions compared with the SAL group ($P < 0.01$ for all comparisons; Fig. 4A-F). In contrast, PD20 treatment significantly reduced TNF- α levels in both the PFC and hippocampus ($P < 0.05$) compared with the SPS group. IL-1 β levels in the hippocampus were also significantly decreased in the PD20 group ($P < 0.05$). PT treatment did not significantly change inflammatory cytokine levels in either the PFC or hippocampus.

Effects of PD and PT-type saponins on BDNF and TrkB mRNA expression in the hippocampus following SPS. After PT or PD treatment in the SPS-induced model of memory impairment, the mRNA expression levels of BDNF and TrkB were analyzed in the hippocampus. The SPS group showed significantly reduced BDNF mRNA expression in the hippocampus compared with the SAL group ($P < 0.01$; Fig. 5). By contrast, PD20 administration significantly increased BDNF mRNA expression in the hippocampus compared with the SPS group ($P < 0.05$). No significant changes in BDNF mRNA expression were observed in the PT-treated group.

Effects of PD and PT-type saponins on the TLR4 and NF- κ B levels in the hippocampus following SPS. To clarify the molecular mechanisms underlying these findings, hippocampal levels of TLR4 and NF- κ B were measured after PT or PD administration in the SPS-induced model of memory impairment. The SPS group showed significantly elevated TLR4 and NF- κ B protein levels compared with the SAL group ($P < 0.01$; Fig. 6). By contrast, PD20 treatment significantly reduced both

proteins in the hippocampus ($P < 0.05$). These results suggest that the anti-inflammatory effects of PD are associated with modulation of the TLR4/NF- κ B signaling pathway.

Discussion

This study compared the effects of PT and PD on PTSD-induced impairments in learning and memory. Unlike PT, PD improved SPS-induced cognitive deficits, reduced pro-inflammatory cytokine production in the PFC and hippocampus, and restored the SPS-induced decrease in BDNF mRNA expression in the hippocampus. These findings suggest that the memory-enhancing and anti-inflammatory effects of PD are associated, at least in part with modulation of the TLR4/NF- κ B signaling pathway.

After SPS exposure, reduced body weight gain and increased serum CORT levels were observed. These changes indicate that the PTSD-like model was successfully established using the SPS paradigm and are consistent with physiological responses commonly associated with acute trauma and chronic stress. Accumulating evidence suggests that dysregulation of the HPA axis leads to sustained elevations in circulating CORT levels, which are closely linked to the development of memory impairments (19).

Several studies have reported that animals exposed to contextual fear paradigms, such as a single traumatic stimulus combined with contextual recall, exhibit enhanced fear conditioning and altered extinction learning (18). In this study, SPS-exposed rats displayed increased freezing behavior during contextual fear conditioning and impaired extinction recall. Short-term extinction recall was also significantly elevated. PD administration attenuated these effects by reducing freezing responses during short-term extinction retrieval.

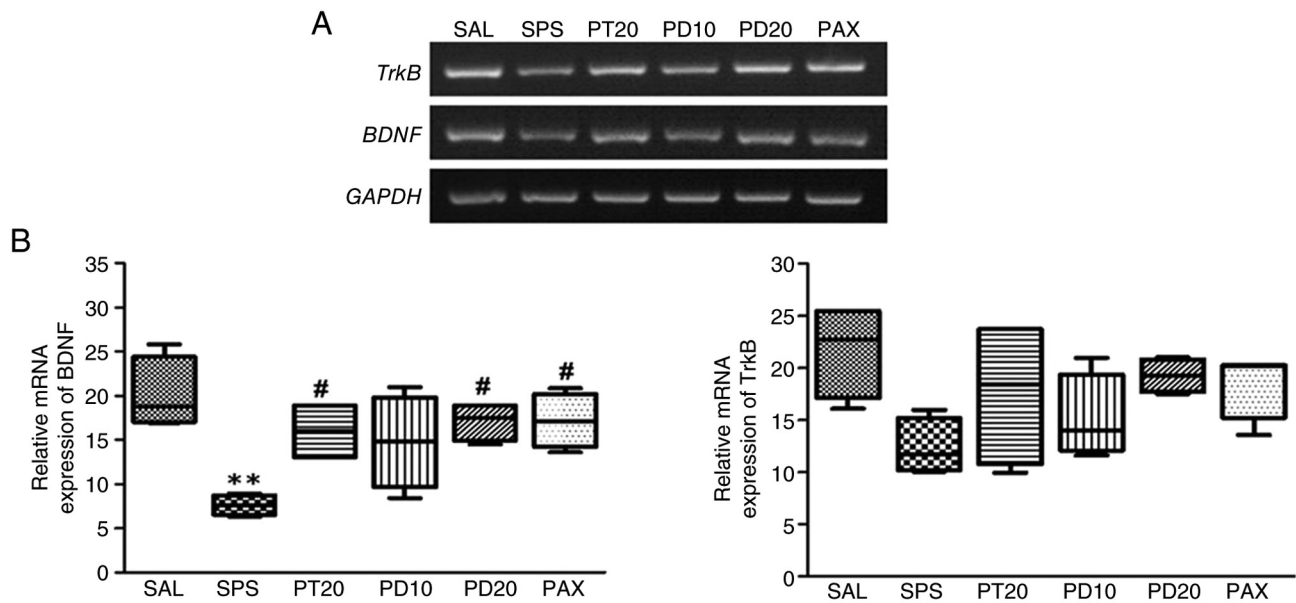


Figure 5. Effect of PD and PT-type saponins on BDNF and TrkB mRNA expression. (A) PCR bands on agarose gels and relative intensities. (B) Comparison of ginsenoside PD or PT effects on the expression of BDNF and TrkB mRNAs in rats with SPS-induced hippocampal impairment. The expression levels of BDNF and TrkB mRNAs were normalized to GAPDH mRNA as an internal control. ** $P < 0.01$ vs. SAL group; # $P < 0.05$ vs. SPS group. BDNF, brain-derived neurotrophic factor; PD, protopanaxadiol; PT, protopanaxatriol; SAL, saline; SPS, single prolonged stress; PAX, paroxetine; TrkB, tropomyosin receptor kinase B.

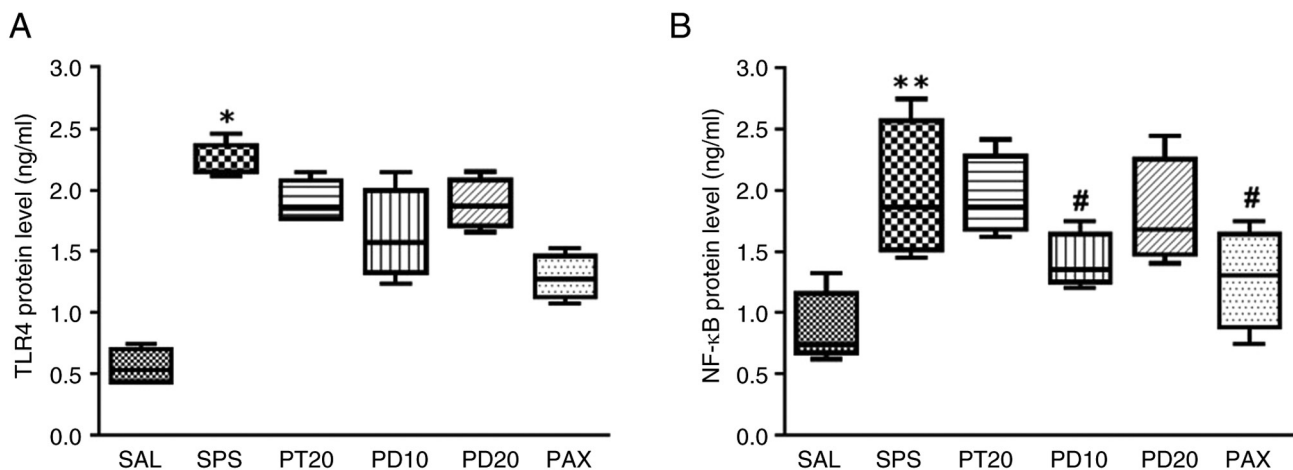


Figure 6. Effect of PD and PT-type saponins on the TLR4 and NF- κ B levels. Comparison of ginsenoside PD or PT effects on (A) TLR4 and (B) NF- κ B levels in the hippocampus of rats exposed to SPS, as measured by enzyme-linked immunosorbent assay. * $P < 0.05$, ** $P < 0.01$ vs. SAL group; # $P < 0.05$ vs. SPS group. NF- κ B, nuclear factor- κ B; PD, protopanaxadiol; PT, protopanaxatriol; SAL, saline; SPS, single prolonged stress; PAX, paroxetine; TLR4, Toll-like receptor 4.

These findings suggest that PD may alleviate maladaptive fear responses associated with traumatic memory, which can interfere with normal memory processing and consolidation. Fear-related memories are known to suppress extinction learning by inhibiting neural activity in key brain regions, including the PFC and hippocampus (20). The present results therefore suggest that PD may contribute to the restoration of impaired fear extinction and memory regulation, potentially through reduced neuroinflammation in the PFC.

SPS exposure also induced in object recognition memory deficits, as shown by reduced novel-object exploration time and decreased discrimination indices (21). These results indicate impaired discrimination between familiar and novel stimuli, reflecting deficits in short-term and reference memory. PD administration significantly improved these cognitive deficits.

SPS-exposed rats exhibited significantly prolonged escape latency in the MWM, indicating impaired spatial learning (22). Deficits in spatial memory consolidation and retrieval were also observed. By contrast, PD administration improved learning performance, as shown by reduced escape latency during the acquisition phase. Spatial memory consolidation was also enhanced, as indicated by increased time spent in the target quadrant and a higher number of platform crossings. These results suggest that PD ameliorates SPS-induced impairments in spatial memory retrieval and recall. Overall, the findings indicate that PD treatment can alleviate SPS-induced deficits in fear memory, spatial learning, and cognitive function. Rapid and persistent neuroinflammatory responses can disrupt memory networks, elevate pro-inflammatory cytokine levels, and alter synaptic plasticity (23,24). Accordingly,

SPS-induced inflammatory responses in the PFC and hippocampus may contribute to the observed cognitive and memory impairments (5). PD administration appeared to exert anti-inflammatory effects by suppressing pro-inflammatory cytokine expression in these brain regions.

BDNF has been reported to promote neuronal survival by modulating NF- κ B signaling cascades and interacting with inflammatory mediators through the BDNF-TrkB pathway (9). Accordingly, BDNF is an important neurotrophic regulator linking neuroinflammation and memory function. Several studies have suggested that modulation of BDNF expression may alleviate neuroinflammation-induced memory deficits in chronic stress and PTSD (25). In this study, PD administration significantly reversed the SPS-induced reduction in BDNF mRNA expression. This effect was accompanied by a decreases in pro-inflammatory cytokines, including TNF- α and IL-1 β , in both the PFC and hippocampus.

TLR4, a key pattern recognition receptor, activates downstream signaling cascades that converge on the NF- κ B pathway, leading to the upregulation of multiple inflammatory mediators (6). After phosphorylation and activation, NF- κ B promotes the expression of pro-inflammatory cytokines, including TNF- α , IL-1 β , and IL-6, thereby contributing to inflammatory responses and tissue injury (6). In this study, SPS-induced PTSD-like animals exhibited elevated levels of TLR4, phosphorylated NF- κ B, TNF- α , and other inflammatory markers. These increases were significantly attenuated after PD administration. These findings suggest that suppression of the TLR4/NF- κ B signaling pathway may underlie the anti-inflammatory effects of PD. Taken together, these results indicate that targeting the TLR4/NF- κ B signaling axis may represent a promising therapeutic strategy for alleviating neuroinflammation and cognitive dysfunction associated with PTSD.

Numerous studies have reported beneficial effects of ginsenosides in animal models of memory impairment induced by chronic stress (26). For example, ginsenoside Rb1, a representative PD-type saponin, has been shown to significantly improve cognitive deficits induced by chronic restraint stress (CRS) in both reward-based instrumental conditioning and MWM tasks. These effects are thought to be mediated through suppression of oxidative stress and apoptosis, thereby enhancing synaptic plasticity (27,28). Similarly, ginsenoside Rg3 has demonstrated neuroprotective properties and cognitive benefits in AD, a condition characterized by excessive accumulation of β -amyloid peptide (A β 1-42) (29). In addition, ginsenoside Rg1, a PT-type saponin, has been reported to alleviate learning and memory deficits in CRS models by preventing reduction in BDNF and TrkB expression in the PFC (30). The PD-type ginsenoside group mainly includes Rb1, Rb2, Rc, and Rd, whereas the PT-type group consists primarily of Rg1, Re, and Rf (13,14). Although both PD- and PT-type ginsenosides have been reported to improve cognitive impairment in various studies (31,32), the present findings indicate that PD-type ginsenosides exert a more pronounced effect on SPS-induced deficits in spatial and cognitive memory. In contrast, PT-type ginsenosides did not produce significant improvements under the same conditions. Taken together, these results suggest that PD-type ginsenosides may be more effective than PT-type ginsenosides in restoring spatial, cognitive, and fear-related memory impairments associated

with SPS-induced PTSD-like conditions, rather than chronic stress alone.

In conclusion, this study demonstrated that PD-type ginsenoside administration improved multiple aspects of memory function, including learning acquisition, memory retention, and fear extinction, in an SPS-induced rat model of memory impairment. These beneficial effects appear to be associated with anti-inflammatory mechanisms involving modulation of the TLR4/NF- κ B signaling pathway. Taken together, PD-type ginsenosides may represent promising bioactive compounds for preventing or alleviating memory decline associated with neuropsychiatric disorders such as PTSD.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

BL performed conceptualization, formal analysis, funding acquisition, investigation, methodology, project administration, visualization and wrote the original draft. BL confirms the authenticity of all the raw data, and read and approved the final manuscript.

Ethics approval and consent to participate

All animal experiments were performed in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals and were approved by the Animal Care and Use Committee of Kyung Hee University (approval no. KHSASP-21-268).

Patient consent for publication

Not applicable.

Competing interests

The author declares that they no competing interests.

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