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The cognitive effect of Protopanaxatriol (Ppt) on memory impairment in mice induced by chronic sleep deprivation

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Abstract
Protopanaxatriol (Ppt) is a neuroprotective ginseng extract and has an antioxidative effect in physiological conditions. The potential neuroprotective effects of Ppt are becoming widely recognized and studied. Sleep deprivation (SD) has been associated with memory impairment through induction of oxidative stress. The present study aimed to investigate the effect of Ppt against chronic sleep deprivation (CSD)-induced cognitive impairment and attempted to define the possible mechanisms involved. We found that 2-week CSD induced significant cognitive impairment in mice evaluated by the object location recognition experiment and Morris water maze test. All of these changes induced by CSD were ameliorated by Ppt (20μmol/Kg; 40μmol/Kg) and Modafinil (0.42g/Kg).In addition, Ppt restored the levels/activities of antioxidant defense biomarkers in the cortex and hippocampus, including glutathione reduced (GSH), glutathione oxidized (GSSG), GSH/GSSG ratio, the superoxide dismutase (SOD) enzyme activity, catalase (CAT), and Lipid peroxidation (LPO).These results suggest that Ppt prevents cognitive impairment induced by CSD. The possible mechanism may be attributed to its ability to reduce oxidative stress in cortex and hippocampus.

Keywords: Protopanaxatriol (Ppt), chronic sleep deprivation (CSD), cognitive impairment, Cortex, hippocampus, oxidative stress

1. Introduction
Sleep is essential for human physiological functioning. It provides restoration of emotional and physical activity in a manner that is not well established [1, 2]. An important function of sleep is the consolidation of new memories into long-term storage sites [3-5]. Several human and animal studies have shown that both acute and chronic sleep deprivation produced memory defects in a number of behavioral tasks [6-9]. Additionally, sleep deprivation elevated oxidative stress in the hippocampus, which reflects on cognitive functions [10-15]. Sleep deprivation as cognitive challenge may provide a promising preclinical model of MCI and a useful tool to study cognition enhancing drugs [16]. *Panax ginseng* C.A. Mayer, an ancient and well-known herbal drug in traditional Chinese medicine, is a perennial herb of the family *Araliaceae*. A large number of reports have demonstrated that *panax ginseng* possesses a number of beneficial effects in the CNS [17, 18]. Protopanaxtriol (Ppt) is a neuroprotective ginseng extract and has an antioxidative effect in physiological conditions. It was shown to reduce learning and memory impairment associated with Alzheimer's diseases [19] and protect against 3-NP-induced oxidative stressin the rat model of Huntington's disease, which is associated with its anti-oxidant activity [20]. In the current study, the possible preventive effect of ppt on impairment of memory induced by chronic sleep deprivation was examined. Possible involvement of antioxidant properties of ppt in such effect was also investigated.

2. Materials and methods
2.1 Apparatus and reagents
Morris water maze, object recognition test system and sleep interruption apparatus were all developed jointly by China astronaut research and training center and Chinese Academy of Medical Sciences (CAMS) Institute of Medicinal Plant Development (IMPLAD). Ppt (purify >98% by HPLC) was purchased from Ruifensi biological technology co. (Chengdu, China). Modafinil was purchased from Xiangbeiweierman co. (Guangzhou, China), GSH, GSSG, SOD, CAT and LPO commercial kit were obtained from Jiancheng biological technology co. (Nanjing, China).

2.2 Animals and treatment protocol

Sixty ICR male mice (weighing 20-22g, Vital river, Beijing, China) were housed at (25 ± 2) ℃ under 12-h light and dark cycles, and allowed access to food and water ad libitum. The experiment was carried out according to the "Principles of Laboratory Animal Care" (NIH publication No.86-23, revised in 1996) and P. R. China legislation for the use and care of laboratory animals.

Animals were assigned to five groups randomly (12 animals in each group): Control group (Con), Sleep deprivation model group (SD), SD+ Ppt (min group, 20μmol/kg; max group, 40μmol/kg) groups and SD+ Modafinil group (MOD, 0.42g/kg). Ppt and MOD were dissolved in the physiological saline (0.9%, Nacl. N.S) by ultrasound. The animals in each group received physiological saline (Con and SD group), various concentrations of Ppt (20μmol/kg; 40μmol/kg) and Modafinil (0.42g/kg) until the end of the behavioral test. All drugs and physiological saline were administrated by intraperitoneal injection (20ml/kg) once daily for 2 weeks. The behavioral tests were carried out immediately after 2 weeks of treatment. SD and/or Ppt treatments continued throughout the behavioral testing days.

2.3 Induction of Sleep Deprivation

Briefly, the SD mice were prepared by subjecting the mice to our self-made Sleep Interruption Apparatus (SIA, a computer controlled rotating drum which has been applied for Chinese patent; patent No., 201210356645.X) continuously for 14 days (24h/d). The mice were free access to water and food in the SIA during the 14-days SD. The parameters of SIA were set as follows: rotation speed, 60 seconds per revolution; rotation frequency, rotating one minute after 2-min pause. Mice in the control group were housed in a static SIA copy as those used in the SD mice [21].

2.4 Object location recognition experiment

The task took place in object recognition test system with 40 cm×50 cm closed field surrounded by 50 cm high walls, made of test chamber with back polyester plastic material. Headlamp with LED are located on both sides of cabinet, for avoiding the interference of strong light on animal behavior, and also acting as background light to contribute to identification of animal. A camera is mounted on the ceiling, and used to observe animal's movement and exploratory behavior. This experiment process could be divided into 3 stages: habituation, familiar and test phase. In habituation phase (3 days), mice were exposed to arena alone (no objects) in turn to freely explore the surroundings 10 min for reducing animals' fear of new environment. In the fourth day, the experimental trials began, which consisted of a familiar phase and test phase with 30 min interval between each trial. During the interval mouse was placed in the holding cage, which remained inside the testing room. During familiar phase, mice were placed in arena which contained two copies of object (A1 and A2) and allow to freely explore (5 min per trail). After 30 min delay period, a test trail was conducted; mice were returned to arena which one of original object changed placement ("novel") and anther object remained ("familiar"). Objects and their placement into the arena were varied across mice to avoid positional biases. Objects were placed in the center of the arena approximately 10 cm from the arena wall and held in place with magnet. To control for possible odor cues, the objects and the floor of the arena were cleaned with 70% ethanol at the end of each trial to eliminate possible scent/trail markers.

Exploratory behavior was considered only when the mice were sniffing or touching the object with his nose. In each trail, duration of contacting with each object was recorded using a stopwatch. The video of animal exploratory behavior was recorded using video camera, and all behaviors were scored from video to ensure accuracy. Recognition memory was evaluated using a discrimination index (DI) calculated for each animal using the formula: DI = (TN-TF)/(TN+TF) corresponding to the difference between the time exploring the novel and the familiar object, corrected for total time exploring both objects TN, exploration time of "novel" object; TF, exploration time of "familiar" object. In familiar phase, the total exploration time (Te) and exploration time for A1 and A2 object were also recorded.

2.5 Morris Water maze task

Morris water maze task（MWM）was conducted after object location recognition experiment to evaluate the spatial memory in mice. For the acquisition test, mice were repeatedly placed into the tank and must learn to locate a hidden platform (6cm in diameter) beneath in water. The water maze was consisted of circular, black pool measuring 1.0 m in diameter×0.38 m in height, and filled with opaque water (black ink) at the temperature of (23±1) ℃ to a depth of 25 cm. A video camera monitored the behavior of the mice in the pool, allowing measurement of a number of parameters, including escape latency, average speed, swimming distance in target quadrant and swimming time in target quadrant. The test room contained several permanent extra cues such as lights, a picture on the wall, etc. Acquisition test consisted of five consecutive daily trails in which mice were placed (facing the wall) three times into each of four quadrants in turn (except for target quadrant), and the order was unchanged each day. Before each trail, mice were placed on the hidden platform for 10s. If mice did not locate the safe platform within 90 s, the mice were gently guided to the platform, allowing mice to stay the platform 10 s, and the escape latency was recorded 90 s. The inter-trial time was approximately 90 s. On the day following completion acquisition testing, animals were probed in a 90 s "retention" trail in which the platform was removed (probe test). The mice were placed in the pool side opposite to the target (platform) quadrant. The swimming distance and time in target quadrant were compared with time in other quadrants, and virtual-platform crossing numbers were recorded. Higher percentage of swimming distance and time in target quadrant and virtual-platform crossing numbers were used as an index of memory retention.

2.6 Tissue dissection

After behavioral tests, blood samples of the mice were collected from the right orbital vein, and the brains were excised immediately after killing the mice under anesthesia with 10% chloral hydrate. The entire cortex and hippocampus were dissected from the whole brain on ice, separated from the midsagittal plane, and immediately snap-frozen and stored at 80 °C until further analysis.

2.7 Calorimetric enzymatic assays

To determine activities or levels of oxidative stress enzymes and molecules, cortex and hippocampus tissues were homogenized manually using small pestle in lysis buffer (137 m MNaCl, 20 m MTris–HCl pH 8.0, 1% nonylphenyl polyethylene glycol ether with 20 molecules ethylene oxide, 10% glycerol, 0.5 m M sodium vanadate, 1 m Polymethylene

sulfonyl fluoride), and protease inhibitor cocktail (Sigma–Aldrich Corp, MI, USA). Homogenates were centrifuged to remove insoluble material (14,000 × g for 5 min, 4 ◦C). Total protein concentration was estimated using commercially available kit (Bio RAD, Hercules, CA, USA). To quantify glutathione (GSH), homogenates were deproteinized in 5% 5-sulfosalicylic acid, centrifuged at 10,000 × g and 4 ◦C for 10 min, and then the supernatants were assayed for total GSH and GSSG according to manufacturer instructions (Glutathione Assay Kit, Jiancheng, Nanjing, China). For analysis of GSSG, 10 L of 1 M 4-vinylpyridine (Sigma–Aldrich Corp) was added per 1 mL of supernatant. GSH was calculated by subtracting total glutathione species value from GSSG value. Catalase, superoxide dismutase (SOD) and LPO activities were measured using commercially available kits according to manufacturer's instructions (Jiancheng, Nanjing, China).

2.8 Statistical Analysis

All data are expressed as mean ± standard error of the mean (SEM). The data was carried out using one-way analysis of variance (ANOVA). Following significant ANOVAs, multiple post hoc comparisons were performed using the LSD test. The data recorded from the acquisition trails of MWM among the groups over a period of 5 days were analyzed with repeated measures and a multivariate analysis of variance (ANOVA) process of the general linear model. Statistical significance was set at $p < 0.05$ in all of the evaluations. The analysis was done using SPSS 19.0 for Windows (Chicago, Illinois, USA).

3. Results

3.1 Effects of chronic sleep deprivation and Ppt on the object location recognition task

We tested the effects of Ppt on recognition memory using object location recognition task (Fig.1). In familiar phase, analysis of the performance shows that there were no clear differences between treatment conditions in the level of exploration (Fig.1A). Paired Samples t-tests indicated that differences were not found for the exploration time of "familiar" object and "novel" object comparing between groups (Fig.1C). It suggested that there were no differences for animals' ability of exploration and preference for location. The effects of Ppt (ip pre-training) on the relative discrimination index (DI) are presented (Fig.1B). When comparing between groups, differences were found for the DI [F (4, 40) =7.536, p<0.0001]. Post hoc analysis indicated the DI is higher in the Ppt 20μmol/kg (p<0.001), Ppt 40μmol/kg (p<0.001), MOD 0.42g/Kg(p＜0.001)and vehicle condition (p<0.001) than in the SD group condition. It illustrated that Ppt 20μmol/kg, Ppt 40μmol/kg and Modafinil 0.42g/Kg could reverse the chronic sleep deprivation induced recognition memory deficit.

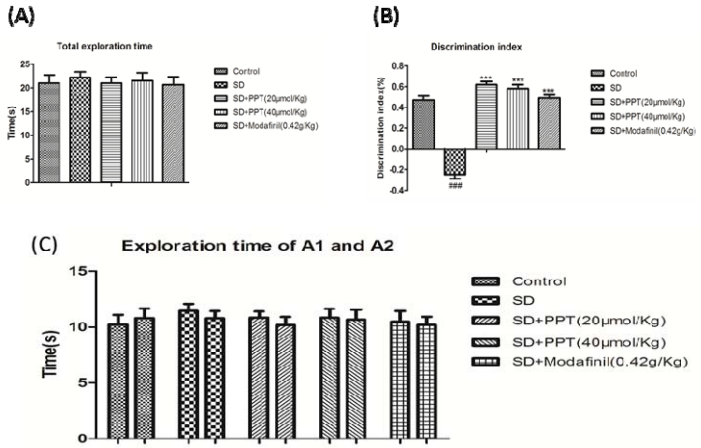

Fig 1: Effect of Ppt on spatial recognition memory in OLR task on mice induced by CSD. (A) Total exploration time, (c) exploration time of A1 and A2 respectively of mice in habituation phase. Subpart (B) stands for the discrimination index in test phase. Data are expressed as means ± SEM (n=12). Significant differences ***p<0.001 compared with the SD group; ###p<0.001 compared with control group

3.2 Effects of chronic sleep deprivation and Ppt on spatial learning of acquisition in mice

Effects of Ppt on spatial learning and memory performance were assessed using Morris water maze task (MWM). In the navigation phase, mice had to learn to locate a hidden platform through distinguish visual cues in the test room. Daily data on spatial learning are shown (Fig.2A-D). The latency of acquisition decreased progressively during the training days in all groups. We first used two-way repeated measures ANOVA to analyze the interaction effects between groups and days of training. The statistical analysis results revealed that the group had a significant effect on the escape latency ($F_{4,245}$=4.413,p=0.001) in navigation phase. And also significant day effect on the escape latency ($F_{4,245}$=23.616,p<0.001) were observed. Likewise, there was a significant interaction between two factors (day ×group) in escape latency ($F_{16,245}$=2.090, p=0.017) and no significant interaction in swimming time ($F_{16,245}$=1.089,p=0.372) and distance spent in target quadrant($F_{16,245}$=0.992,p=0.463) during 5 days training. Sleep deprivation group performed significantly fewer latency with shorter swimming time and distance in the target quadrant than control group, indicating that the ability of navigation was impaired after chronic sleep deprivation. After treatment with Ppt, the impaired navigation ability was improved.

Moreover, following significant ANOVAs, we analyzed the differences between groups on each day using the LSD test. We found a significant differences between SD model group and control group on escape latency (day3, p=0.028; day4, p=0.010; day 5, p<0.001). Also significant differences were observed in SD model group vs. control group on swimming distance in the target quadrant (day2, p=0.032; day4, p=0.003) and swimming time in the target quadrant (day 4, p=0.006; day 5, p=0.011). Ppt (20μmol/kg) attenuated the

spatial learning deficits by CSD, as indicated by a reduction of the escape latency (day 3, p=0.003; day 4, p=0.004; day 5, p<0.001), swimming speeding (day 4, p=0.041) and swimming time in target quadrant (day 4, p=0.044; day 5, p=0.024). Significant differences were found in Ppt (40μmol/kg) vs. SD model group on escape latency (day 3, p=0.011; day 4, p=0.017; day 5, p=0.014) and swimming time in target quadrant (day 4, p=0.024). Modafinil (0.42g/Kg) improved the spatial learning deficits by CSD on escape latency (day 2, p=0.011; day 3,p=0.005;day 4, p=0.008; day 5, p=0.001) and swimming time in target quadrant (day 2, p=0.041; day 5, p=0.030).

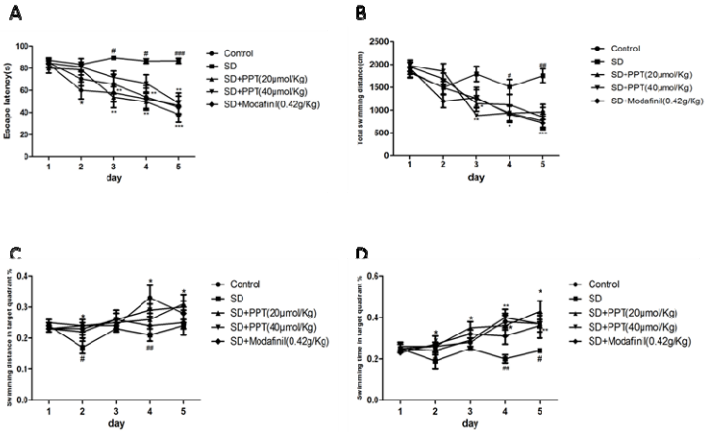

Fig 2: Effect of Ppt on the acquisition of spatial reference memory in MWM task on mice induced by CSD. (A) Escape latency, (B) Total swimming distance, (C) swimming distance in target quadrant and (D) swimming time in target quadrant of mice to hidden platform across 5 days of training trials (3 trials per day). Data are expressed as means ± SEM (n=12). Significant differences *p<0.05, **p<0.01, ***p<0.001 compared with the SD model group; #p<0.05, ##p<0.01, ###p<0.001 compared with control group.

3.3 Effects of chronic sleep deprivation and Ppt on spatial learning in probe trial in mice

A probe trail with the platform unavailable was conducted for these five groups of mice after acquisition test. Fig.3A showed that virtual-platform crossing numbers were obviously reduced in SD model group, indicating that CSD impaired the formation of long-term memory in mice. After treated Pptip administration for 14 days, CSD induced spatial memory index, such as virtual-platform crossing numbers, swimming distance in target quadrant and time in target quadrant, had been improved obviously. This showed that Ppt could ameliorate the impaired spatial memory induced by CSD. We analyzed the differences between groups on each day using one-way ANOVA as follows.

We had found that significant differences of virtual-platform crossing numbers ($F_{4,46}$=3.477, p<0.006) in SD model group vs. control group. The swimming distance and swimming time in target quadrant were also decreased in SD model group, but no statistical difference (p>0.05). Significant differences were found in Ppt (20μmol/kg) group on time in target quadrant ($F_{4,46}$=1.713, p<0.033) and distance in target quadrant ($F_{4,46}$=1.664, p<0.031). Performance of spatial memory in probe test in Ppt(40μmol/kg) group mice were better than SD model group on virtual-platform crossing numbers ($F_{4,46}$=2.151, p=0.022); time in target quadrant ($F_{4,46}$=1.850, p=0.027) and distance in target quadrant ($F_{4,46}$=1.604, p<0.031).The virtual-platform crossing numbers in Modafinil (0.42g/Kg) group were significantly increased ($F_{4,46}$=1.225, p=0.006), at the same time, the swimming distance and swimming time in target quadrant were also increased, but no statistical difference (p>0.05) compared with SD model group.

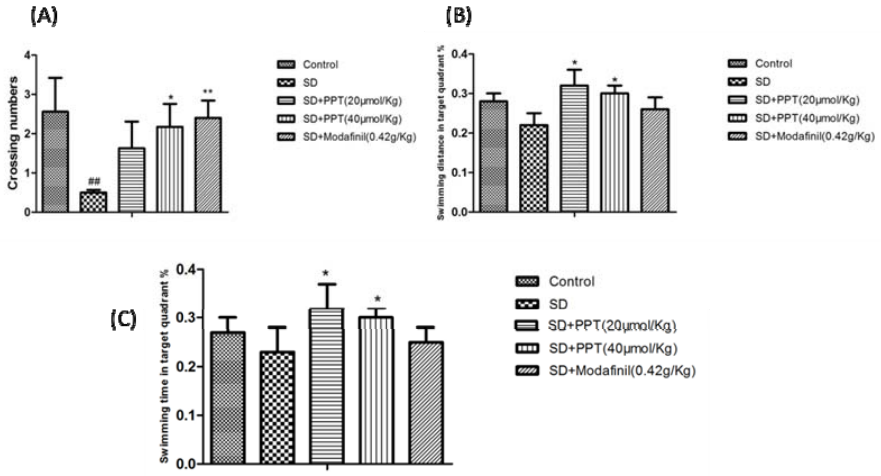

Fig 3: Effect of Ppt on memory retention in probe test of MWM task on mice induced by CSD. The probe test was carried out 24h after the acquisition phase. (A) Virtual-platform crossing numbers, (B) swimming distance in target quadrant, (C) swimming time in target quadrant, Data are expressed as means ± SEM(n=12). Significant differences *p<0.05, **p<0.01, compared with the SD model group; #p<0.05, ##p<0.01, compared with control group.

3.4 The effect of chronic sleep deprivation and/or Ppt on cortex and hippocampus oxidative stress markers/antioxidant enzymes

3.4.1 Levels of the GSH, GSSG and GSH/GSSG ratio

Levels of GSH in the SD group were significantly decreased while treatment with Ppt could elevate it in the cortex and hippocampus (Fig. 4A). No significant difference was observed in GSSG levels among all the experimental groups in the cortex, while in the hippocampus levels of GSSG in the SD group were increased significantly, at the same time, Ppt (40μmol/kg) group and Modafinil (0.42g/Kg) group could decrease the level of GSSG (Fig. 4B). A significant decrease in the levels of GSH resulting in a significant decrease in GSH/GSSG ratio, were detected in the SD group compared to other experimental groups (Fig. 4C). Thus, treatment with Ppt and Modafinil protected cortex and hippocampus from chronic sleep deprivation induced reduction of GSH and elevation of GSSG.

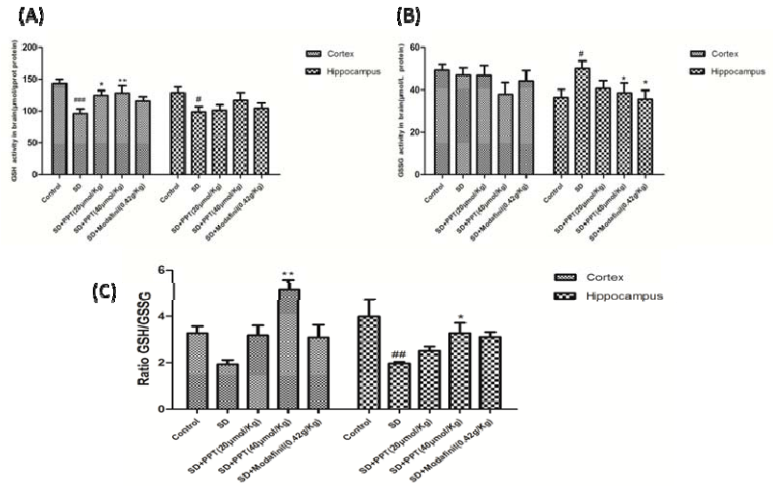

Fig 4: Effect of Ppt on glutathione levels in the cortex and hippocampus of mice induced by CSD. (A) Levels of reduced glutathione (GSH). (B) Levels of oxidized glutathione (GSSG). (C) Ratio of GSH/GSSG. Both GSH and GSSG were measured using commercially available glutathione assay kit. Data are expressed as means ± SEM (n=12). Significant differences *p<0.05, **p<0.01, compared with the SD model group; #p<0.05, ##p<0.01, compared with control group.

3.4.2 Superoxide dismutase (SOD) activity

After CSD, the SOD activity in the cortex and hippocampus was significantly reduced compared to the control group (P < 0.01; Fig. 5). On the other hand, a significant increase in the level of SOD in cortex and hippocampus were observed in SDs mice by administering Ppt, indicating that Ppt restored SOD activity in the cortex and hippocampus, which is reduced during chronic sleep deprivation.

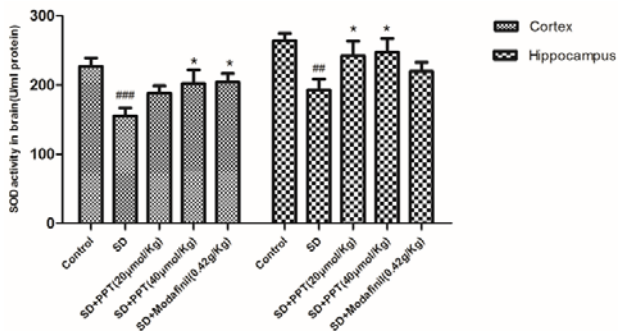

Fig 5: Effect of Ppt on SOD enzymatic activity in the cortex and hippocampus of mice induced by CSD. Chronic sleep deprivation is associated with reduction in the cortex and hippocampus activity of SOD, which was recovered by Ppt administration. Data are expressed as means ± SEM (n=12). Significant differences *p<0.05, compared with the SD model group; #p<0.05, ##p<0.01, ###p<0.001 compared with control group.

3.4.3 Catalase (CAT) activity

Chronic sleep deprivation significantly reduced the activity of catalase in the cortex and hippocampus compared to control group (P < 0.05; Fig. 6). On the other hand, the activity of catalase in Ppt (20μmol/kg)、Ppt (40μmol/kg)、Modafinil (0.42g/Kg) groups were elevated compared to SD group, indicating that Ppt recovered the cortex and hippocampal activity of catalase, which is reduced during chronic sleep deprivation.

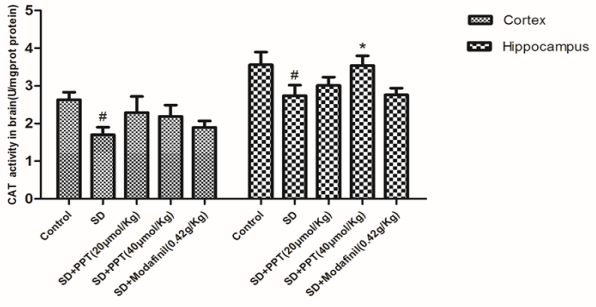

Fig 6: Effect of Ppton Catalase enzymatic activity in the cortex and hippocampus of mice induced by CSD. Chronic treatment with Ppt restored activity of catalase during chronic sleep deprivation. Catalase was measured using commercially available kits according to manufacturer's instructions. Data are expressed as means ± SEM (n=12). Significant differences *p<0.05, compared with the SD model group;#p<0.05, compared with control group.

3.4.4 Levels of Lipid peroxidation (LPO)

Chronic sleep deprivation significantly elevated the levels of lipid peroxidation in the cortex and hippocampus compared to control group (P < 0.01; Fig. 7). On the other hand, the levels of LPO in Ppt (20μmol/kg)、Ppt (40μmol/kg)、Modafinil (0.42g/Kg) groups were significantly reduced compared to SD model group, indicating that Ppt recovered the cortex and hippocampal levels of LPO, which is reduced during chronic sleep deprivation.

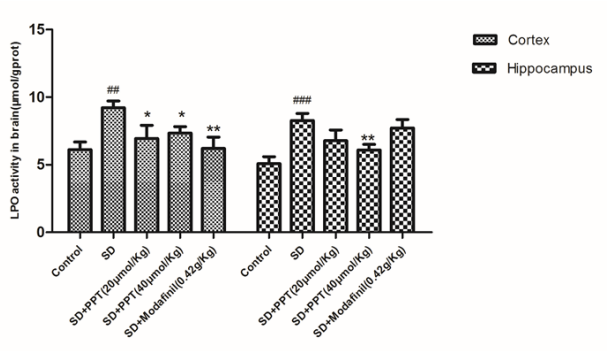

Fig 7: Effect of Ppt on levels of lipid peroxidation in the cortex and hippocampus of mice induced by CSD. Chronic treatment with Ppt restored activity of lipid peroxidation during chronic sleep deprivation. Data are expressed as means ± SEM (n=12). Significant differences *p<0.05, **p<0.01, compared with the SD model group; #p<0.05, ##p<0.01, ###p<0.001 compared with control group.

4. Discussion

In the present study, we investigated the memory-enhancing effect of Ppt in CSD model, using object location recognition task (OLR) and Morris water maze task (MWM) to assess behavior, and measured the levels of oxidative stress markers including GSH, GSSG, SOD, CAT and LPO activity in the cortex and hippocampus in mice. The results showed that Ppt treatment at 20μmol/kg and 40μmol/kg per day plays a significant neuroprotective role in repairing the cognitive impairment induced by CSD.

Sleep is regarded as a brain state that enhances the removal of neurotoxic waste products and promote learning-related synapse formation and maintenance, which consequently is critical for brain function, especially the cognitive performance [22, 23]. Sleep loss was previously associated with memory impairment in both human and animal studies. Numerous studies found that sleep deprivation (SD) alters the neuronal excitability in the cognition-related brain regions and induces impairment of cognitive performance in rodents and humans, especially in hippocampus-dependent tasks [13, 14].

Object recognition task is based on the principle of the nature of preference for novel objects in rodent, could sensitively assess animal behavior of the object recognition memory ability in the spontaneous state [24]. During the experiment, the animals were in accordance with characteristics, location, order of appearance, and background of objects to distinguish the different "identity" objects, achieving the transition through detection of simple non-spatial memory to complex spatial, temporal and episodic memory [25, 26]. Our present study showed after one of the two copies objects which mice were familiar with changed its location, the chronic sleep deprived mice did not distinguish the "novel" object (changed location) relative to "old" object (unchanged location), and a significant reduction of the discrimination index (DI) which was used to evaluated recognition memory in CSD model group was found compared to Con group. Furthermore, we investigated that ip administration (20μmol/kg and 40μmol /kg) for Ppt could significantly ameliorate the impaired object location recognition memory in CSD model mice. Fig.1B showed that the discrimination index (DI) in Ppt (20μmol/kg and 40μmol/kg) group were significantly increased compared to CSD model group (p<0.001), it is indicated that the mice by ip administration of Ppt (20μmol/kg and 40μmol/kg) could distinguish the "old" object (unchanged location) and "novel" object (changed location), and increase the time to explore the "novel" object. But there were some doubts that whether this effect was caused by the differences of exploration ability or preference for a particular location in mice. So the total exploration time (Te) and exploration time for A1 and A2 object in familiar phase were recorded. Statistics showed that there were no significant differences between all groups in the level of exploration, and paired samples T-test revealed that differences were not found for exploration time for A1 and A2 object comparing between groups. This suggested that there were no differences for animal's capacity of exploration and preference for a particular location. Taken together, it is likely that Ppt ameliorated the object location recognition memory in mice induced by CSD, which reflects spatial short-term memory.

To further verify the effects of the Ppt by ip administration on spatial learning and memory, we examined the mice using MWM. MWM is general accepted as an indicator of spatial learning and reference memory, which reflects long-term memory [27]. Between 1 and 5 days in the hidden-platform training, mice in the SD model group showed significant spatial learning impairment, mainly manifested in Fig.2A which showed that escape latency in SD group were obviously higher than Con group. Ppt (20μmol/kg and 40μmol/kg) significantly shorten the escape latency after 2 days training. The swimming distance and time spent in the target quadrant in which the platform had been placed in the training trials is shown in Fig.2C-D. The swimming distance and time in the target quadrant of SD model group was significantly shorter than the Con group respectively on day2, day 4; day4, day5. Ppt (20μmol/kg and 40μmol/kg) significantly increased the swimming distance and time in the target quadrant after 2 days training. In addition, during the probe trial, Ppt group (20μmol/kg and 40μmol/kg) also significantly increased the swimming distance and time in the target quadrant (Fig.3B-C). It is reported that virtual-platform crossing numbers when the platform was absent in probe trial is an key indicator assessed reference memory. Fig.3A showed that Ppt (40μmol/kg) significantly increased the virtual-platform crossing numbers compared to SD model group. Given all this, we could see that Ppt (20μmol/kg and 40μmol/kg) improved the CSD mice reference memory.

Experimental studies have shown that Modafinil has beneficial effects on cognitive tasks such as attention and working memory in experimentally sleep-deprived humans, primates, and rodents [28, 29]. In according with the previous studies, our results showed that treatment with Modafinil (0.42g/Kg) could improve the performance in object location recognition task (OLR) compared to SD model group. In addition, the acquisition of MWM was significantly better performed in Modafinil (0.42g/Kg) group, and in probe test, Modafinil (0.42g/Kg) group showed significant effect on the improvement of spatial memory. In addition, Modafinil (0.42g/Kg) group exhibited antioxidation to some extent in the study.

Oxidative stress plays a crucial role in impairment of cognitive processes in several health conditions such as aging [30], traumatic brain injury [31], and Alzheimer's disease [32]. An imbalance between reactive oxygen species and antioxidant enzymes may lead to oxidative stress state [33]. The SOD enzyme catalyses the dismutation of superoxide ion into hydrogen peroxide, which is then neutralized by catalase [9, 34]. The glutathione system includes both enzymatic (e.g., glutathione peroxidase) and non-enzymatic (e.g., GSH) antioxidants [35]. When GSH is oxidized, it will be transformed to the oxidized form (GSSG), which transforms a hydrogen peroxide molecule into a water molecule. These mechanisms are crucial to reduce oxidative stress in the brain

and related neuron dysfunction, cognitive impairment, and the resulting spatial learning and memory impairments [36-38]. Numerous studies have shown that both acute and chronic SD increases oxidative stress in hippocampus and other brain areas [15, 39]. In addition, some studies have reported increased oxidative stress in the hippocampus and cortex in SD rats [40, 41]. In confirmation, our study confirms that CSD for 14 days caused significant changes in cortex and hippocampal oxidative stress by reducing the activity of GSH, ratio GSH/GSSG, SOD, CAT and increasing the levels of LPO and GSSG. The reduction in antioxidant defenses mechanisms increase oxidative stress which provides a reasonable explanation for the memory deficits following CSD.

Panax ginseng C.A. MEYER (Araliaceae), which contains ginsenosides as its main components, has beenshown to have various biological effects, including anti-inflammatory, anxiolytic, anti-stress, and anti-tumoreffects. Protopanaxtriol (Ppt) is a neuroprotective ginseng extract. Ppt exerts its antioxidative activity via several mechanisms, such as direct scavenging of free radicals and restoration of succinate dehydrogenase activity. These mechanisms may contribute to its potent antioxidative and putative neuroprotective activities [20]. Though the potential neuroprotective effects of Ppt are becoming widely recognized and studied, however, whether Ppt has neuroprotective effect on CSD mice is unknown. In this study, our results showed that ip administration of Ppt to mice prevented chronic sleep deprivation induced memory impairments probably through antagonizing oxidative stress in the cortex and hippocampus.

In conclusion, Ppt prevents short- and long-term memory impairments induced by chronic sleep deprivation probably through antagonizing oxidative stress in the cortex and hippocampus.

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6. Conflicts of Interests

The authors have declared that there is no conflict of interest.

7. References

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