Supporting Information

Silymarin Prevents Memory Impairments, Anxiety, and Depressive-Like Symptoms in a Rat Model of Post-Traumatic Stress Disorder
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Materials and Methods

Induction of PTSD using SPS model

PTSD was induced in the SPS and SPS+silymarin groups (20 rats per group) using the SPS model as reported previously [1,2]. Briefly, each rat was wrapped firmly and immobilized using a plastic bag for 2 h, with the edges covered with tape to prevent the rat from escaping, while a hole for the nose was opened to allow breathing. After the immobilization period, the rats were immediately forced to swim in a cylindrical tank of water for 20 min followed by a 15-min rest. Afterward, the rats were anesthetized until loss of consciousness by exposure to ether for 1–2 min.

RAWM

Spatial learning and memory functions were evaluated using the water maze task model (RAWM) as reported previously [3]. Briefly, a black circular pool containing six swimming arms was filled with water at room temperature. A hidden black platform was submerged about 1 cm below the water level toward the end of one of the swimming arms (goal arm). RAWM testing was conducted in a dimly lit room with two different pictures posted on the room walls to serve as cues for rats. Each rat was randomly placed into one of the arms other than the one containing the platform and allowed to swim to find the escape platform. The rat was given 1 min to locate the platform in both the learning phase and the memory test phase. An error is scored if the rat entered an arm other than the platform arm or if the rat entered the goal arm but did not reach the platform. The rat was guided to the goal arm if it failed to locate the platform within 1 min. The rat was allowed to stand on the platform for about 15 s to observe the room before it was released into the pool for the next trial. The learning phase consisted of a total of 12 trials with a 5-min rest after the first six trials. Short-term memory test was carried out 30 min following the 12th trial, while the long-term memory tests were performed after 5 and 24 h,
respectively. The goal arm was kept the same for each particular rat during the learning phase and the memory phase.

**OFT**

The OFT was used to investigate for anxiety-like behavior as reported previously [4,5]. Briefly, a large cubic box (72 × 72 × 35 cm) opened from the top was used. The bottom surface of the box was divided using a tape into 16 squares (18 × 18 cm). The four middle squares define the central quadrant of the OFT apparatus. The experimental rats were transferred to the OFT room about 30 min before the start of behavioral testing. Each rat was placed beside the wall of the chamber, and its activity while exploring the new environment was monitored for 15 min by placing a camera above the open-field apparatus. The chamber was thoroughly cleaned by alcohol and allowed to dry after each trial. The behavior of the animals was evaluated by measuring the following parameters: ambulatory activity by number of line crossings, freezing time, number of rears, time spent in grooming behavior, time spent in the center, and number of entries to the central area.

**EPM**

Anxiety-like behavior was measured as well using the EPM as described elsewhere [6]. Briefly, a four-armed plus-shaped platform raised about 50 cm from the ground was used. Two opposing arms have walls from three sides (protected area) while the other two arms were left open (unprotected area). The rats were transferred to the EPM experimental room 30 min prior to the start of the first trial. Each rat was placed in the central neutral area (10 × 10 cm) with its head positioned toward the open arm. The rat’s movement for about 5 min was observed using a camera that was placed above the platform. The EPM platform was wiped with alcohol and allowed to dry after each trial. A set of both conventional and ethological parameters were
obtained: time spent in both the protected and unprotected areas, % time spent in the protected and unprotected areas, the number of entries to the protected and unprotected areas, % of entries to the protected and unprotected areas, and the number of returns to the protected, unprotected, and neutral areas. An entry is counted if the rat enters an area with its four paws. Nevertheless, a return is counted if the rat explores the other area by head extension with only forepaws inside and then withdraws completely back to its original area.

TST

The TST was performed to measure depression-like behavior as reported previously [5]. Briefly, the rats were suspended up in the air from their tails. The tails of the rats were fixed at a bench edge using tape along with plastic climbing stoppers to prevent tail-climbing. Actions were taken to prevent rats from observing each other during the TST. The rat’s movement for about 6 min was recorded using a camera that was placed in front of the rat. The recorded video of each rat was analyzed for immobility time. Immobility was defined as the lack of movement in the animals’ hind legs. Movements in the front legs without the activity of the hind legs or movements due to the resulting momentum from prior mobility were not counted as mobility.
References


