Supporting Information

Pharmacokinetic-Pharmacodynamic (PK-PD) Modeling of Effect of Naringenin and Its Surface Modified Nanocarriers on Associated and Core Behaviors of Autism Spectrum Disorders (ASD)

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S1. Behavioral Tests

a. Rotarod test for sensorimotor dysfunction

This test is used to assess motor coordination and balance alterations in rodents. It was first introduced by Dunham and Miya to study neurological functioning and was later improved to investigate motor deficits of naïve rats and the effects of drug administration. Rotarod rotates at a speed of 20 rpm. Locomotor activity was observed for a period of 300 s, which was the cutoff time. Time of fall(s) was observed [1].

b. Actophotometer

In order to detect the association of neurological functions with changes in motor activity, the locomotor activity of rats was tested by a digital actophotometer (IMCORP). A total number of ambulatory movements and rearing were scored in the 5-min test period to evaluate locomotor activity [2].

c. Open-field activity test

It is used for assessment of anxiety, as indicated by a number of entries in the center circle and from the number of line crossings. Open-field apparatus is a modification of the usual square shaped box apparatus. It is a wooden circular arena of 84 cm in diameter, painted white on the floor, and divided into 1 bigger circle that was divided into 16 equal parts. The main arena contained an inner circle of diameter 38.5 cm, which was further divided into 8 equal parts and contained a central circle of 15 cm diameter. The rat was left in the arena in the center and was
allowed to explore the arena for 5 min. The number of line crossings, rearings, and entries into center circle as well as the frequency of urination and defecation were recorded [3].

d. Reciprocal social interaction

This was used to test the ability of a rat induced with ASD-like phenotype to socially interact with a rat from another home cage. Reciprocal social interaction was observed between 2 rats, one an unfamiliar male rat from other home cage and another one from each group. Various parameters were observed for social as well as nonsocial interaction. Parameters for social interaction that were scored include one mouse walks closely behind the other, keeping pace and push-crawl (physical contacts include pushing the snout or head underneath the partner’s body, squeezing between the partner and the arena wall or floor, and crawling over or under the partner’s body). Parameters for nonsocial interaction include self-grooming (the rat grooms its face and body regions in a normal sequential pattern) and arena exploration (walking around the arena, sniffing the walls, floor and bedding, and digging the bedding). Total time spent in social and nonsocial interactions, respectively, was separately measured in a 10-min session [4].

e. Repetitive self-grooming test

Rats show spontaneous motor stereotypies including circling, jumping, and self-grooming. Repetitive self-grooming was observed in rats induced with ASD-like phenotype by placing them in the social arena and after sprinkling some light water mist on their back and neck area. Total time spent in grooming head or rest of the body during the 10-min session was recorded [5].
References