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ARRIVE STUDY CHARACTERISTICS

According to the ARRIVE 2.0 Guidelines
https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.3000411

ITEM	
Study design	Experiment 1 (for data in figures 1-3) a. There are five groups: sham, vehicle, and three groups injected with genistein at the dose of 5, 10, 20 mg/kg b. The experiment unit: single animal Experiment 2 (for data in figures 4-5) a. There are three groups: sham, vehicle, and the groups injected with genistein at the dose of 20 mg/kg b. The experiment unit: single animal Experiment 3 (for data in figure 6) a. There are three groups: sham, vehicle, and the groups injected with genistein at the dose of 20 mg/kg b. The experiment unit: single animal
Sample size	a. Exact number of experimental units allocated to each group and total number in each experiment. For the behavioral tests and brain water examination, a total of 60 rats with 12 rats per group were used. For the following experiments, a total of 36 rats with 12 rats per group were used. Total number of rats used: 96 b. The sample size was decided based on the previous publication.
Inclusion and exclusion criteria	a. No criteria were set a priori for the inclusion or exclusion of animal. b. There were no exclusions c. For each analysis, the exact value of n in each experimental group was specified in the corresponding figure legends.
Randomisation	The rats were randomly allocated to the experimental groups.
Blinding	The researchers were blind to the group allocation.
Outcome measures	a. Neurological deficit evaluation b. Locomotion activity examination by open-field test c. Anxiety and explorative behaviors by elevated plus maze test d. Brain water content e. Apoptosis in the brain f. Inflammatory activities in the brain
Statistical methods	All data were expressed as the mean with standard deviation (mean ± SD). One-way ANOVA followed Dunn's multiple comparisons test were used. $P < 0.05$ was considered statistically different. The significance levels were denoted as: ***$p < 0.001$ compared to sham. ^$p < 0.05$, ^^$p < 0.01$ and ^^^$p < 0.001$ compared to Vehicle.
Experimental animals	Adult male Sprague-Dawley (SD) rats with the weight of 300-350 g were used. A total of 96 rats were used.
Experimental procedures	TBI models were constructed using adult male Sprague-Dawley (SD) rats with the weight of 300-350 g using the Lateral Fluid Percussion Injury (LFPI) method for inducing both focal and diffusive injuries to the brain. A total of 87 rats were used. Briefly, the animals were fixed on a stereotaxic table, routinely disinfected and toweled, and the skin and periosteum were cut in layers in the middle of the head to

	expose the right parietal bone, and the rat's brain was positioned at 3 mm next to the sagittal suture and 3.5 mm behind the coronal suture. A hole was drilled in the skull with a craniotomy drill to expose the meninges, and a small cap of the same size as the hole was placed on the dura mater. After fixing the hydraulic tube, 3 atm (standard for heavy craniocerebral injury) was administered, and the sham operation group only opened the bone window without impact.
Results	Experimental 1 (mean/SD): Sham, Vehicle, Gen5, Gen10, Gen20: Fig1A: 34.83 18.33 22.17 24.50 28.42 4.324 2.270 3.243 4.232 4.144 Fig1B: 31.14 19.57 23.83 25.23 27.25 3.809 3.633 3.453 3.905 4.020 Fig1C: 15.65 8.111 10.06 11.22 13.32 1.637 1.568 1.769 2.071 1.937 Fig1D: 43.25 98.86 84.57 75.65 60.12 7.689 12.14 8.159 10.33 8.775 Fig2A: 26.82 42.25 38.84 34.56 31.45 5.107 6.891 6.110 5.876 5.668 Fig2B: 12.76 6.231 7.815 8.720 11.11 2.113 1.227 1.207 1.738 1.668 Fig2C: 0.4075 0.2317 0.2800 0.3158 0.3675 0.07412 0.04529 0.03464 0.05696 0.07485 Fig2D: 24.37 13.24 16.13 17.31 19.46 4.558 2.309 2.138 2.566 2.615 Fig3B: 78.12 84.73 82.13 81.46 80.14 1.314 1.790 1.399 1.611 1.589 Experimental 2 (mean/SD): Sham, Vehicle, Gen20: Fig4B: 2.300 32.41 17.35 0.3162 3.572 2.102 Fig5A: 21.13 161.8 78.98 3.650 12.91 17.83 Fig5B: 15.65 128.4 61.22 3.106 18.68 15.23

	Fig5C: 35.21 215.7 119.9 5.450 27.99 18.25 Experimental 3 (mean/SD): Sham, Vehicle, Gen20: Fig6A: 0.9997 4.364 1.743 0.07365 0.2112 0.1154 Fig6B: 1.000 3.273 1.835 0.08581 0.1755 0.1205 Fig6C: 1.000 3.423 2.111 0.09102 0.1884 0.1431 Fig6E: 1.000 4.289 2.113 0.08551 0.1968 0.1268 Fig6F: 1.000 4.699 2.487 0.1024 0.3149 0.2787 Fig6G: 1.000 3.142 2.164 0.09558 0.2410 0.1465 Fig6H: 0.9997 3.683 1.795 0.1034 0.2754 0.1615

Abstract	Objective: Traumatic brain injury (TBI)-induced anxiety is a common but under-investigated disorder, for which neuroinflammation is a significant contributor. Here we aim to investigate the protective effects of genistein, a plant-derived anti-inflammatory drug, against TBI-induced anxiety, and the underlying mechanisms. Methods: A rat model of TBI that exhibited anxiety-like behaviors was constructed using the Lateral Fluid Percussion Injury (LFPI) method. Genistein at the doses of 5, 10, and 20 mg/kg were used to treat rats at 30 min, 12 h, 24 h, 48 h, and 72 h up to 14 days after TBI. The evaluation of neurological deficit was performed preoperatively, on days 1, 3, 7, and 14 after TBI. The elevated plus maze (EPM) test was carried out on day 12 after TBI to assess anxiety and explorative behaviors, and the open field test (OFT) was performed on day 13 after TBI to assess locomotive activities. Brain injury was assessed by measuring brain water content and TUNEL staining. Inflammatory responses were examined by measuring interleukin (IL)-1β, IL-18, and tumor necrosis factor α in the lesioned cortices tissues using ELISA assay. The changes of mRNA and protein expression in the Nlrp3/caspase-1 signaling was analyzed using real-time PCR and Western blot, respectively. Results: In the behavioral level, genistein treatment alleviated TBI-induced anxiety and neurological deficit in animals demonstrated in EPM and OPF experiments. In the meanwhile, brain edema was also reduced by genistein treatment, showing alleviating effects of genistein in the pathological level. TUNEL staining also showed reduced apoptosis in rats treated by genistein. Genistein also inhibited Nlrp3/caspase-1 signaling, unveiling the effects of genistein in altering molecular pathways in brains with TBI. Conclusion: Genistein alleviates anxiety-like behaviors in rats with TBI, which may be mediated via inhibiting Nlrp/caspase-1 signaling pathway.
Background	a. Traumatic brain injury (TBI), which is the third most common cause of death worldwide, is

	characterized by non-degenerative, acquired structural damage and/or brain dysfunction caused by an external force, leading to alterations in the level of consciousness, and long-term or transient disability of cognitive and physical functions. Neuropsychiatric disorders, including anxiety, depression, and psychosis, are common consequences of TBI. Owing to advances in treatment strategies, there is a significant decrease in mortality rate of TBI, but a tremendous gap exists in the clinical management of neuropsychiatric detriments caused by TBI. b. Inflammation is a central driver of TBI-induced psychiatric impairments. Following the direct mechanical brain damage, irreversible neuronal death occurs, which comprises the primary damage caused by TBI. Subsequently, a cascade of molecular, biochemical and cellular changes, including predominantly inflammatory responses and free radical generation, was initiated that further exerts deleterious effects on neuron physiology for hours after TBI, which comprises secondary damage by TBI. Hence, recent studies have largely explored anti-inflammatory drugs to reduce TBI-induced brain inflammation. One of the anti-inflammatory drugs commonly used for neurological disorders is genistein, a plant-derived isoflavone found in soy foods. The potent effects of genistein in reducing neurological damage have been demonstrated in its role in alleviating ischemic injury in the brain, stroke, Alzheimer's disease, etc. For example, it was shown that genistein delays the onset of mortality and disability in a model of amyotrophic lateral sclerosis. A previous study also provided a preliminary testing showing that genistein can be neuroprotective in TBI. In this study, genistein was shown to attenuate brain edema, blood-brain barrier (BBB) disruption, and aberrant neurobehavioral performances. However, whether genistein treatment resulted in amelioration of TBI-induced neuropsychiatric disorders remains unknown.
Objectives	The current work aimed to assess the therapeutic potential of genistein on anxiety behavior in animals after TBI.
Ethical statement	As detailed as follows: Animal studies were approved by the ethics committee of Xingtai Medical College (#2023-XTMC-288).
Housing and husbandry	The rats were housed in groups of 2 per cage.
Animal care and monitoring	a. There were no unexpected or adverse events. b. The study did not have humane endpoints.
Interpretation/ scientific implications	a. The results suggest that Genistein was effective in alleviating anxiety-like behaviors in rat with TBI, and this effect could be mediated by the role of genistein in inhibiting NLRP3/caspase-1 signaling. b. Main limitation of the study: Only TBI animal model was employed in the current study, other animal models of brain injury rather than TBI could be used. The detailed molecular mechanisms underlying the protective effects of genistein could be further explored using omics, such as RNA-sequencing.
Generalisability/ translation	The finding may translate to other rodents and humans.
Protocol registration	No protocol was registered.
Data access	The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.
Declaration of interests	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.
Abstract	Objective: Traumatic brain injury (TBI)-induced anxiety is a common but under-investigated disorder, for which neuroinflammation is a significant contributor. Here we aim to investigate the protective effects of genistein, a plant-derived anti-inflammatory drug, against TBI-induced anxiety, and the underlying mechanisms. Methods: A rat model of TBI that exhibited anxiety-like behaviors was constructed using the Lateral Fluid Percussion Injury (LFPI) method. Genistein at the doses of 5, 10, and 20 mg/kg were used to treat rats at 30 min, 12 h, 24 h, 48 h, and 72 h up to 14 days after TBI. The evaluation of neurological deficit was performed preoperatively, on days 1, 3, 7, and 14 after TBI. The elevated plus maze (EPM) test was carried out on day 12 after TBI to assess anxiety and explorative behaviors, and the open field test (OFT) was performed on day 13 after TBI to assess locomotive activities. Brain injury was assessed by measuring brain water content and TUNEL staining. Inflammatory responses were examined by measuring interleukin (IL)-1β, IL-18, and tumor necrosis factor α in the lesioned cortices tissues using ELISA assay. The changes of mRNA and protein expression in the Nlrp3/caspase-1 signaling was analyzed using real-time PCR and Western blot, respectively. Results: In the behavioral level, genistein treatment alleviated TBI-induced anxiety and neurological

	deficit in animals demonstrated in EPM and OPF experiments. In the meanwhile, brain edema was also reduced by genistein treatment, showing alleviating effects of genistein in the pathological level. TUNEL staining also showed reduced apoptosis in rats treated by genistein. Genistein also inhibited Nlrp3/caspase-1 signaling, unveiling the effects of genistein in altering molecular pathways in brains with TBI. Conclusion: Genistein alleviates anxiety-like behaviors in rats with TBI, which may be mediated via inhibiting Nlrp/caspase-1 signaling pathway.Conclusion: Genistein was effective in alleviating anxiety-like behaviors in rat with TBI, and this effect could be mediated by the role of genistein in inhibiting NLRP3/caspase-1 signaling.