PI3K/AKT/mTOR signalling inhibitor chrysophanol ameliorates neurobehavioural and neurochemical defects in propionic acid-induced experimental model of autism in adult rats

Aarti Sharma1 · Sonalika Bhalla1 · Sidharth Mehan1*

Received: 15 November 2021 / Accepted: 5 June 2022 / Published online: 10 June 2022 © The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

Abstract
Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder marked by social and communication deficits as well as repetitive behaviour. Several studies have found that overactivation of the PI3K/AKT/mTOR signalling pathways during brain development plays a significant role in autism pathogenesis. Overexpression of the PI3K/AKT/mTOR signalling pathway causes neurological disorders by increasing cell death, neuroinflammation, and oxidative stress. Chrysophanol, also known as chrysophanic acid, is a naturally occurring chemical obtained from the plant Rheum palmatum. This study aimed to examine the neuroprotective effect of CPH on neurobehavioral, molecular, neurochemical, and gross pathological alterations in ICV-PPA induced experimental model of autism in adult rats. The effects of ICV-PPA on PI3K/AKT/mTOR downregulation in the brain were studied in autism-like rats. Furthermore, we investigated how CPH affected myelin basic protein (MBP) levels in rat brain homogenate and apoptotic biomarkers such as caspase-3, Bax, and Bcl-2 levels in rat brain homogenate and blood plasma samples. Rats were tested for behavioural abnormalities such as neuromuscular dysfunction using an actophotometer, motor coordination using a beam crossing task (BCT), depressive behaviour using a forced swim test (FST), cognitive deficiency, and memory consolidation using a Morris water maze (MWM) task. In PPA-treated rats, prolonged oral CPH administration from day 12 to day 44 of the experimental schedule reduces autistic-like symptoms. Furthermore, in rat brain homogenates, blood plasma, and CSF samples, cellular, molecular, and cell death markers, neuroinflammatory cytokines, neurotransmitter levels, and oxidative stress indicators were investigated. The recent findings imply that CPH also restores abnormal neurochemical levels and may prevent autism-like gross pathological alterations, such as demyelination volume, in the rat brain.

Keywords Autism · PI3K/AKT/mTOR · Chrysophanol · Propionic acid · Cell death · Neuroinflammation

Introduction
Autism spectrum disorder (ASD) is a severe neurodevelopmental illness characterised by stereotyped or repetitive behaviour, memory and cognitive dysfunctions associated with sensory and motor function impairments (Gvozdjáková et al. 2014; Neumeyer et al. 2019). Early childhood ASD symptoms appear shortly after birth, resulting in lifelong disabilities (Jin et al. 2015). Neurochemical alterations trigger Autism-like repetitive and stereotypical behaviours in specific brain regions, including the hippocampus, cerebellum, amygdala, and cerebral cortex (Khera et al. 2022a, 2022b; Lee et al. 2016; Morimoto et al. 2020; Sacai et al. 2020). Previous research has found that a subgroup of autistic children has gastrointestinal symptoms and aberrant gut microbiota (Kang et al. 2018; Finegold et al. 2012).

Autistic patients have increased numbers of the bacteria Clostridium and Desulfovibrio, which are known to produce short-chain fatty acids such as Propionic acid (PPA) (Finegold 2011). Propionic acid (PPA) is a short-chain fatty acid that readily crosses the gut–blood barrier and can enter the CNS via the monocarboxylate transporter system (Mirza and Sharma 2019; Shams et al. 2019). PPA accumulates inside cells, causing intracellular acidification, mitochondrial malfunction (Mehan et al. 2020), increased oxidative stress markers (Rahi...
et al. 2021), altered neurotransmitters (Tiwari et al. 2021), and impaired synaptic transmission in autistic rat brains (Meeking et al. 2020). Sharma et colleagues found that stereotaxic PPA injection into the neocortex area promotes stereotyped behaviour and neurochemical changes in experimental models of autism in adult rats (Sharma et al. 2019).

The PI3K/AKT/mTOR signalling pathway is primarily involved in neural activities such as synaptic plasticity, neuronal development, memory consolidation, and protein synthesis (Kassai et al. 2014; Takei and Nawa 2014). It also affects a variety of physiological and biological processes, such as neuronal growth, axon guidance, cell proliferation, and differentiation (Rai et al. 2019; Jafari et al. 2019).

Upregulation of the PI3K/AKT/mTOR signalling pathway has also been associated with cerebral cell proliferation, axonal disruption, and megalencephaly in various brain regions (Kim and Guan 2015; Subramanian et al. 2015; Hutsler and Zhang 2010). According to previous research, the PI3K/AKT/mTOR pathway governs translation in dendritic spines, and its activation increases dendritic spine malfunction, leading to autism-like symptoms (Ganesan et al. 2019; Soltani et al. 2017; Maiti et al. 2015). Overexpression of the PI3K/AKT/mTOR pathway resulted in learning and memory problems (Sharma and Mehan 2021), social impairment (Costa-Mattioli and Monteggia 2013), and abnormal synaptic plasticity (Li et al. 2010). The PI3K/AKT/mTOR signalling pathway has also been linked to the pathophysiology of several neurodevelopmental and neuropsychiatric disorders, including depression (Neis et al. 2020), cognitive development-associated brain malformation (Rivière et al. 2012a, 2012b), and epilepsy (Rivière et al. 2012a, 2012b). (Xiao et al. 2015; Brandt et al. 2018).

The PI3K/AKT/mTOR pathways are activated in the development of several neurodegenerative illnesses, including Huntington’s disease (Abd-Elrahman and Ferguson 2019), Alzheimer’s disease (Hodges et al. 2018), and brain trauma (Xu et al. 2020). Furthermore, motor neuron illnesses such as Multiple Sclerosis (Giacoppo et al. 2017) and Parkinson’s disease are caused by the overactivation of the PI3K/AKT/mTOR pathway (Chen et al. 2019).

Chrysophanol (CPH) is a 1, 8-dihydroxy-3-methyl derivative of the 9, 10-anthracenedione ring identified in Rheum rhubarbarum, a herbaceous perennial plant in the Polygonaceae family (Singh et al. 2013). CPH has a wide range of pharmacological effects and biological activities, including anti-depressant, anti-bacterial, and anti-cancer properties (Rokaya et al. 2012; Su et al. 2020). CPH also has antimicrobial, anti-inflammatory, and antiviral effects (Lian et al. 2017) and is used to treat a variety of neurological dysfunctions (Chaet et al. 2017; Jeong et al. 2018). CPH has been shown in preclinical investigations to alleviate cognition deficits and neuronal death in streptozotocin-induced diabetic encephalopathy (Chu et al. 2018). CPH has been demonstrated to protect against neurodegenerative diseases affecting the motor neurons, including multiple sclerosis (MS) (Lee et al. 2016) and Parkinson’s disease (PD) (Chae et al. 2017). A recent preclinical study has investigated the neuroprotective potential of CPH via PI3k/Akt/mTOR pathway in intracerebral haemorrhage (Jadaun et al. 2022a, 2022b). To summarise CPH’s relationships with possible targets, Wang and Lv validated CPH’s interaction with mTOR against malignant meningioma by blocking mTOR signalling and increasing NF2 signalling (Wang and Lv 2021). Previous research on colorectal cancer (Deng et al. 2020) and colon cancer (Lee et al. 2011) found that CPH reduced PI3K/AKT/mTOR levels, reducing pathological conditions.

Based on the findings, we hypothesise that CPH can downregulate the abnormal PI3K/AKT/mTOR signalling mechanisms, reducing the neuropathological abnormalities in an ICV-PPA-induced experimental model of autism in adult rats. As a result, the current study looked at the overexpression of PI3K/AKT/mTOR, which is involved in the pathogenesis of autism. We investigated the neuroprotective effect of CPH on behavioural, neurochemical, and morphological characteristics in an ICV-PPA-induced experimental model of autism in adult rats. Thus, CPH provides neuroprotection in autistic rats by downregulating the PI3K/AKT/mTOR signalling pathway, which was proven by examining neurochemical parameters in biological samples such as CSF, blood plasma, and brain homogenates.

**Materials and methods**

**Experimental animals**

A total of 36 rats were used in the current study. All experiments were conducted on six-month-old adult Wistar rats’ weight 250–300 g. Each group contains six rats, either sex; they were obtained from the Central Animal House, ISF College of Pharmacy, Moga, Punjab, India. Animals were housed in an acclimatized environment with a 12-hour light-dark cycle with food and water at room temperature at 23 ± 2 °C. The Institute for Animal Ethics Committee (IAEC) approved the project as 816/PO/ReBiBt/S/04/CPCSEA as IAEC/CPCSEA/Meeting No: 27/2020/Protocol No. 454, following the guidelines provided by the government of India. The rats were randomly divided into six groups, and the sample size was based on a validated animal sampling method suggested by Charan and Kantharia 2013. Animals were acclimatized to laboratory conditions before experimentation.
Drugs and chemicals

PPA was purchased from Sigma–Aldrich (USA). CPH was provided as an ex-gratia sample from BAPEX, India. All other chemicals utilized in the experiments are of analytical grade. Before using the drugs and chemicals, fresh solutions were prepared. CPH was given orally by dissolving in an aqueous solution of 2% ethanol (Chu et al. 2018). The dosing of chrysophanol was determined based on previous research findings in various brain diseases, including ischemic brain injury (Zhao et al. 2016a, 2016b), learning and memory deficits (Dong et al., 2010), cognition deficits and neuronal loss (Chu et al. 2018), and Cerebral Ischemia (Zhang et al. 2014a, 2014b).

Experimental grouping of animals

The total duration of the experimentation was of 44 days. Propionic acid (PPA) was injected from day 1st to day 11th into the intracerebroventricular (ICV) region of the rat brain to induce autism. CPH was administered orally from day 12th to day 44th. Animals were randomly assigned into six groups. Group 1 - vehicle control; Group 2 - sham control; Group 3 - Chrysophanol perse (20 mg/kg., p.o.); Group 4 - PPA (10 μl/0.26 M, i.c.v.); Group 5 - PPA (10 μl/0.26 M, i.c.v.) + Chrysophanol (10 mg/kg., p.o.); Group 6 - PPA (10 μl/0.26 M, i.c.v.) + Chrysophanol (20 mg/kg., p.o.). The present study was unblinded, and the experimenter was known regarding the care of animals. All behavioral parameters were conducted from day 1 to day 44th. On day 45th, after completing the protocol schedule, the blood plasma, CSF was collected from adult Wistar rats. Besides, Sodium pentobarbital 270 mg/ml, i.p., was used to anaesthetize the animals deeply. After anaesthetization, the fresh brain was removed and preserved with ice-cold PBS (0.1 M) PBS for further neurochemical analysis. The experimental protocol is summarized in (Fig. 1).

ICV-PPA induced experimental model of autism in adult rats

The PPA-induced experimental model of Autism in rats was established and validated by Tiwari et al. 2021. Experimental rats were treated with a PPA-ICV injection of 10 μl/0.26 M for consecutive 11th days. The study by Rahi et al. 2021 suggests that PPA causes behavioral and neurochemical alteration similar to an experimental animal model of autism and is regarded as a validated experimental model for researching the pathophysiological changes identical to those seen in Autistic patients.

Rats were allowed to be habituated in a laboratory environment. Eventually, rats were anaesthetized by intraperitoneal injection of 75 mg/kg ketamine. Then, the rats were placed on the stereotaxic instrument (Stoelting Co., Wood Dale, IL, USA) in a skull-flat position. The positioning of the head was adjusted prior to the surgery to ensure that the bregma and lambda coordinates were similar and at the same level. The rats’ heads were shaved, the scalp had been cleaned with 70% ethanol and incised with a blade (mid-sagittal), the skin was removed, and the skull was exposed to spot bregma and lambda that was indicated to assist in defining ICV injection coordinates. Wet cotton swabs were put on rat eyes to prevent dehydration, and cotton buds were used to stop bleeding. A hole was drilled in the skull (Stereotaxic coordinates: AP $= -1.3$ mm; ML $= -1.8$ mm; DV $= -3.0$ mm), a cannula inserted in the burr hole, and the cannula was closed using a plastic ear-pin. The dental
cement was filled in the hole and then sutured with an absorbable surgical suture attached to the sterile surgical needle (González-Fraguela et al. 2013).

For post-operative care, rats were kept independently in a polyacrylic cage that contained warm cloth. Special care was needed until they restored spontaneous movement, which occurred approximately 2–3 hours after anaesthesia. The room temperature was set at 25 ± 3 °C. For 2–3 days, milk and glucose water were provided inside the cages to avoid physical trauma after surgery. To prevent sepsis, gentamycin (35 mg/kg) was given intraperitoneally for three days, and lignocaine gel was applied to the sutured area to relieve the pain. Neosporin powder was sprinkled on them to prevent bacterial infection of the skin. After surgery, the body’s overall health and clinical symptoms such as dehydration, body weight, infection, and other physical changes were closely monitored.

Parameters

Measurement of weight variations

Assessment of body weight The body weight was measured on the 1st, 13th, 23rd, 33th, and 43rd days of the experiment protocol schedule (Sharma et al. 2019; Sahu et al. 2022).

Measurement of relative brain-body weight ratio The relative brain-body weight ratio was calculated on the 45th day of the experimental protocol schedule (Khera et al. 2022a, 2022b; Gopi et al. 2019).

Behaviour parameters

Morris water maze task (MWM) The Morris water maze test was conducted to evaluate memory and cognitive impairment (Morris 1984). Escape latency time (ELT) was measured using MWM on the protocol schedule’s 40th, 41st, 42nd, and 43rd. Time (seconds) taken by rats to reach the target platform was considered as escape latency. On day 44th, rats were exposed to swim in the pool containing a hidden platform; for 120 seconds, and time spent in the target quadrant (TSTQ) was recorded. The TSTQ represents the degree of memory consolidation, which occurred after learning (Yadav et al. 2022; Duggal et al. 2020).

Locomotor activity The locomotor activity was performed on the 1st, 13th, 23rd, and 43rd days of the experimental protocol schedule using an actophotometer (INCO Group of Companies Dubai, United Arab Emirates). The behaviour parameter was evaluated using the method described by Mehan et al. 2018. The animal was placed in a digital actophotometer equipped with infrared photocells. They are then observed for five minutes in a square, the closed arena. The value of a digital actophotometer begins as counts per 5 minutes (Tiwari et al. 2021; Mehan et al. 2018).

Beam crossing task (BCT) The beam crossing task was conducted on days 1st, 13th, 23rd, and 43rd of the experimental protocol schedule to evaluate motor coordination. During each trial, the number of foot slips was recorded, and additionally, the direction of an animal’s fall was observed against the cut-off time of five minutes (Khera et al. 2022a, 2022b; Sharma et al. 2019).

Forced swim test (FST) The forced swim test was used to measure the depressive-like behaviour of rats on the 1st, 13th, 23rd, and 43rd days. The first exposure of rats in the tank during the training phase is for 15 minutes, and the second is after 24 hours later for 5 minutes. A single six-minute exposure is used during the testing session. The first two minutes serve as a habituation period, with the final four minutes serving as the test itself, which determines the length of immobility (Minj et al. 2021).

Neurochemical parameters

Collection and preparation of biological samples

Blood plasma collection and separation On day 45th of the protocol schedule, anaesthetized the rats with the chloroform before sample collection. Immediately after anesthetization, a capillary tube is placed at the medial canthus of the eye, and then the sinus is ruptured. Instantly 1–2 ml of blood was collected from the rats through retro-bulbar puncture (Sahu et al. 2022; Kumar et al. 2017). The obtained blood samples were then cold centrifuged at 10,000×g for 15 minutes to separate the plasma. Then separated plasma was carefully stored at −80 °C deep freezer for biochemical analysis.

CSF collection Rats were deeply anaesthetized with 270 mg/ml sodium pentobarbital through i.p. injection. The rats’ head was fixed using a holder to reveal the Arachnoid membrane and a skin incision was made, and a translucent dura mater was exposed. A maximum volume of 100 μL CSF was obtained by direct inserting a 30-gauge needle at a 30° angle into the cisterna magna. Within 20 minutes after collection, the sample was centrifuged at 2000 g for 10 minutes at 4 °C. After centrifugation, the supernatant was stored at −80 °C until further analysis (Kozler et al. 2015).

Brain homogenate preparation Rats were sacrificed by decapitation on the 45th day of the treatment schedule. The
whole fresh brain was removed, washed with ice-cold iso-
tonic saline solution, and homogenized with 0.1 M (w/v) of chilled phosphate buffer saline (pH = 7.4). The rat brain homogenate was then centrifuged at 10,000×g for 15 minutes, the supernatant was separated, and the aliquots were preserved. The samples were stored in a deep-freezer at −80 °C to be used as and when the need for various neuro-
chemical estimations (Rahi et al. 2021).

Assessment of cellular and molecular markers

Estimation of PI3K, AKT and mTOR protein level  PI3K protein level was measured in rat brain homogenate (David et al. 2015) and CSF (Tong et al. 2020) using an ELISA kit. According to the instruction given by ELISA assay kits (E-EL-H1019/PI3K; Elabsciences, Wuhan, Hubei, China). The AKT level was estimated in rat brain homogenate (Schipper et al. 2000) and CSF (Dong et al. 2018; Jadaun et al. 2022a, 2022b) (E-AB-21082/AKT; Elabsciences, Wuhan, Hubei, China), mTOR level was measured in rat brain homogenate (Khera et al. 2022a, 2022b; Wang and Zhang 2017) and CSF (Li et al. 2015) samples (E-EL-H1655/mTOR; Elabsciences, Wuhan, Hubei, China).

Estimation of MBP level  The MBP levels were assessed in rat brain homogenate using an ELISA kit (E-EL-R0642/MBP; Elabsciences, Wuhan, Hubei, China). The values were expressed in μg/mg protein (Rahi et al. 2021).

Assessment of apoptotic markers

Caspase-3 concentrations were assessed in brain homogenate (Rahi et al. 2021) and blood plasma (Guo and Li 2018) using an ELISA kit. Bax protein level was determined in brain homogenate (Tiwari et al. 2021) and blood plasma (Wang et al. 2019). The anti-apoptotic protein such as Bcl-2 levels was estimated in brain homogenate (Sharma et al. 2019) and blood plasma (Wang et al. 2019) using ELISA commercial kits (E-EL-R0160/Caspase-3; E-EL-R0098/Bax/Bcl2Elabsciencies, Wuhan, Hubei, China). The values are expressed in ng/g protein in brain homogenate and ng/ml in blood plasma.

Assessment of neurotransmitter levels

Measurement of acetylcholine (ach) level  Acetylcholine level was measured using a diagnostic kit (E-EL-008Ach; ELabSciences, Wuhan, Hubei, China). All samples and reagents were freshly prepared as per manual instruction provided by the kit’s. The optical density of the reaction mixture was measured at 540 nm. The neurotransmitter in the supernatant was estimated and the value expressed as ng/mg protein (Rajdev et al. 2020).

Measurement of dopamine level  The dopamine levels in the striatal tissue sample were determined. Dopamine levels in the striatum are a sign of neural excitability, which leads to mood changes. The electrochemical detector was used to assess dopamine levels in the brain homogenate using the HPLC technique. The level of dopamine in brain homogenates is expressed as ng/mg protein (Gupta et al. 2022; Sharma et al. 2021).

Estimation of glutamate level  Glutamate was assessed after derivatization with o-phthalaldehyde-/−mercap-
toethanol (OPA/−ME), and quantitative analysis in tissue samples using HPLC was carried out according to Rahi and her coworker’s method. The glutamate level in rat brain homogenate is expressed as ng/mg protein (Sharma et al. 2019).

Measurement of serotonin level  Serotonin level was measured in the brain homogenate sample by HPLC using an electrochemical detector and C18 reverse-phase column. The mobile phase consisted of sodium citrate buffer (pH 4.5) – acetonitrile (87:13, v/v). The supernatant was filtered through 0.22 mm nylon filters before being injected into the sample injector. The serotonin concentration was assessed from the standard curve generated using a standard with a concentration of 10–100 mg/ml (Khera et al. 2022a, 2022b; Sharma et al. 2019).

Measurement of inflammatory cytokines levels

The level of TNF was determined in rat brain homogenate (Mehan et al. 2018) and Blood plasma (Fu et al. 2021) using a rat immunoassay kit. The activity of IL-1β was assessed in rat brain homogenate (Minj et al. 2021) and blood plasma (Nwangwu et al. 2017) (E-EL-R0019/TNF-α; E-EL-R0012/IL-1β; ELabSciences, Wuhan, Hubei, China) and value expressed as pg/mg protein (Yu et al. 2018).

Evaluation of oxidative stress markers

Estimation of acetylcholinesterase (AChE) level  Acetylcholinesterase concentration was estimated using spectro-
photometrically. The assay mixture contained 0.05 ml of supernatant, 3 ml of 0.01 M sodium phosphate buffer (pH 8), 0.10 ml of acetylthiocholine iodide, and 0.10 ml DTNB (Ellman reagent). The change in absorbance was recorded instantly at 412 nm spectrophotometrically. The enzymatic activity in the supernatant was expressed as μM/mg protein (Deshmukh et al. 2009).

Measurement of superoxide dismutase (SOD) enzymatic activity  SOD activity was measured using spectrophotometrically by auto-oxidation of epinephrine at pH 10.4. The
supernatant (0.2 ml) of the brain homogenate was mixed with 0.8 ml of 50 mM glycine buffer, pH 10.4, and the reaction was initiated with the addition of 0.02 ml epinephrine. After 5 minutes, the absorbance was spectrophotometrically measured at 480 nm. SOD activity was quantified as nM/mg protein (Sahu et al. 2022; Mehan et al. 2017).

**Estimation of GSH level** The level of reduced glutathione in the brain was assessed using the method described by Ellman et al. 1959. 1 ml supernatant was precipitated with 1 ml of 4% sulfosalicylic acid and cold digested at 4 °C for one hour. The samples were centrifuged at 1200xg for 15 min. To 1 ml of the supernatant, 2.7 ml of phosphate buffer (0.1 M, pH 8) and 0.2 ml of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) were added. The yellow colour appeared immediately measured with a spectrophotometer at 412 nm. The glutathione concentration in the supernatant was expressed as μM/mg protein (Yadav et al. 2022; Bala et al. 2015).

**Estimation of nitrite level** The nitrite concentration in the supernatant, indicating the formation of nitric oxide (NO) is evaluated by a colorimetric assay using a Greiss reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide, and 2.5% phosphoric acid) as described by Green et al. 1982. Equal volumes of supernatant and Greiss reagent are mixed, the mixture incubated for 10 min at room temperature in the dark, and the absorbance determined spectrophotometrically at 540 nm. The amount of nitrite in the supernatant is determined from a sodium nitrite standard curve and expressed as μM/mg protein (Mehan et al. 2020).

**Estimation of malondialdehyde (MDA) level** The quantitative determination of malondialdehyde (MDA) was performed in brain homogenate. After its reaction with thiobarbituric acid, the concentration of MDA was measured at 532 nm using a spectrophotometer and expressed as nM/mg protein (Singh et al. 2021; Sharma et al. 2021).

**Protein estimation** The protein content was quantified by using the Coral protein estimation kit (Biuret method).

**Gross pathological examination of rat brains**

On the 43rd day, rats were sacrificed by decapitation; brains were removed for gross pathological analysis performed. After analyzing the whole brain, coronal sections were taken (Tiwari et al. 2021). Sectioned 2-mm thick brain pieces (coronally from the anterior pole to the posterior poles of the cerebral cortex) were placed on glass slides. A digital camera (Fujix digital camera, Fujifilm, Japan) was used to visualize all the brain regions. The demyelination region (mm) in each brain segment was measured on day 43rd after completing the procedure through MOTICAM-BA310 image plus 2.0 analysis software. The demyelination scale (mm) volume was calculated for each coronal brain segment by converting the demyelination region (mm). The demyelination size (mm3) in each brain section was measured from the dark greyish area near the striatum by image analysis on the 43rd day. The injury’s size was calculated in each coronal 2-mm-thick brain section by calculating the demyelination area (l×b×h) (Khera et al. 2022a, 2022b; Rahi et al. 2021).

**Statistical analysis**

Data were analyzed using two-way ANOVA followed by Post hoc test Bonferroni and one-way ANOVA repeated measures followed by Post hoc test Tukey’s multi comparison test. P < 0.001 was considered statistically significant. Data was found to be normalized, and the sample size was calculated by checking the normality distribution by the Kolmogorov Smirnov test. All statistical results were performed out by GraphPad Prism version 5.03 for Windows (GraphPad Software, San Diego, CA, USA). Statistical results were expressed as the mean ± standard deviation (SD).

**Results**

**Effect of chrysophanol on weight variations in ICV-PPA induced experimental model of autism in adult rats**

Body weight was measured on the 1st, 13th, 23rd, 33th, and 43rd days of the protocol schedule. There was no significant difference in body weight between any treatment groups prior to treatment beginning. Rats who received daily PPA injections for 11 consecutive days had lower body weight on the 13th day as compared to the vehicle, sham, and CPH 20 perse treated groups. Prolonged oral administration of CPH 10 mg/kg and CPH 20 mg/kg restored body weight significantly on the 23rd, 33rd, and 43rd days when compared to PPA-treated autistic rats [two-way ANOVA: F(20,120) = 157.72, p < 0.001]. On the 43rd day, CPH 20 mg/kg was found to be more effective than CPH 10 mg/kg in successfully restoring body weight. (Fig. 2a).
Fig. 2 (a) Effect of chrysophanol on body weight in ICV-PPA induced experimental model of autism in adult rats. (Values expressed as mean±SD (n=6 rats per group). *p<0.001 v/s vehicle control, sham control, and CPH20Perse; @ p<0.001 v/s PPA; @@ p<0.001 v/s PPA+CPH10 (two-way ANOVA followed by post hoc multiple comparison test Bonferroni). (b) Effect of chrysophanol on relative brain-body weight in ICV-PPA induced experimental model of autism in adult rats. (Values expressed as mean±SD (n=6 rats per group). *p<0.001 v/s vehicle control, sham control and CPH20Perse; @ p<0.001 v/s PPA; @@ p<0.001 v/s PPA+CPH10 (one-way ANOVA followed by Tukey’s multiple comparison test). (c) Effect of chrysophanol on neuromuscular coordination in ICV-PPA induced experimental model of autism in adult rats. (Values expressed as mean±SD (n=6 rats per group). *p<0.001 v/s vehicle control, sham control, and CPH20Perse; @ p<0.001 v/s PPA; @@ p<0.001 v/s PPA+CPH10 (one-way ANOVA followed by Tukey’s multiple comparison test). (d) Effect of chrysophanol on escape latency time in ICV-PPA induced experimental model of autism in adult rats. (Values expressed as mean±SD (n=6 rats per group). *p<0.001 v/s vehicle control, sham control, and CPH20Perse; @ p<0.001 v/s PPA; @@ p<0.001 v/s PPA+CPH10 (two-way ANOVA followed by post hoc multiple comparison test Bonferroni). (e) Effect of chrysophanol on locomotor activity in ICV-PPA induced experimental model of autism in adult rats. (Values expressed as mean±SD (n=6 rats per group). *p<0.001 v/s vehicle control, sham control, and CPH20Perse; @ p<0.001 v/s PPA; @@ p<0.001 v/s PPA+CPH10 (two-way ANOVA followed by post hoc multiple comparison test Bonferroni). (f) Effect of chrysophanol on neuromuscular coordination in ICV-PPA induced experimental model of autism in adult rats. (Values expressed as mean±SD (n=6 rats per group). *p<0.001 v/s vehicle control, sham control, and CPH20Perse; @ p<0.001 v/s PPA; @@ p<0.001 v/s PPA+CPH10 (two-way ANOVA followed by post hoc multiple comparison test Bonferroni). (g) Effect of chrysophanol on memory and cognitive impairment in ICV-PPA induced experimental model of autism in adult rats. (Values expressed as mean±SD (n=6 rats per group). *p<0.001 v/s vehicle control, sham control, and CPH20Perse; @ p<0.001 v/s PPA; @@ p<0.001 v/s PPA+CPH10 (two-way ANOVA followed by post hoc multiple comparison test Bonferroni).

Restoration of relative brain-body weight ratio after chronic administration with chrysophanol

On the final day of the procedure, the relative brain-body weight ratio was assessed. On the 43rd day, there was no significant difference in the relative brain-body weight ratio among all groups. When compared to the vehicle, sham, and CPH 20 perse treatment groups, chronic ICV injection of PPA for 11 days resulted in a substantial reduction in the relative brain-body weight ratio. Long-term CPH 20 mg/kg and CPH 10 mg/kg administration led to an increase in relative brain-body weight ratio on the 43rd day when compared to PPA-treated autistic rats [one-way ANOVA: F(5,25) = 1.218, p<0.001]. CPH 20 mg/kg, on the other hand, significantly restored the relative brain-body weight ratio as compared to the CPH 10 mg/kg treated group (Fig. 2b).

Effect of chrysophanol in the amelioration of neurobehavioral alterations in ICV-PPA induced experimental model of autism in adult rats

Improvement in memory and cognition after chronic administration with chrysophanol

To assess memory and cognitive impairment, the Morris water maze test was used. The escape latency was measured on the 40th, 41st, 42nd, and 43rd days of the experiment protocol schedule. PPA-treated rats have a progressive increase in escape latency time (ELT) as compared to vehicle, sham, and CPH 20 perse treatment rats. Long-term oral CPH 10 mg/kg and CPH 20 mg/kg administration significantly reduced ELT in a dose-dependent manner when compared to the PPA-injected group [two-way
ANOVA: F(15,90) = 19.48, p < 0.001. Furthermore, CPH 20 mg/kg administered to rats reduced ELT more effectively than CPH 10 mg/kg administered to animals (Fig. 2c). On the 44th day of the protocol schedule, the time spent in the target quadrant (TSTQ) was measured. TSTQ in chronic PPA-infused rats is considerably lower than in a vehicle, sham, and CPH 20 perse treated rats. Long-term CPH 10 mg/kg and 20 mg/kg administration enhances TSTQ in a dose-dependent manner compared to PPA-treated autistic rats [one-way ANOVA: F(5,25) = 6.594, p < 0.001]. CPH 20 mg/kg-treated rats improved TSTQ and memory consolidation more than CPH 10 mg/kg-treated rats (Fig. 2d).

### Improvement in locomotion after chronic administration with chrysophanol

Rat locomotion was observed using locomotor activity. The actophotometer device was used to perform the test on the 1st, 13th, 23rd, and 43rd days. On the first day of the protocol schedule, there were no significant differences between the treatment groups. PPA-injected rats had significantly lower locomotion on the 13th day compared to the vehicle, sham, and CPH 20 perse treated groups. In comparison to PPA-treated autistic rats, persistent oral treatment of CPH 10 mg/kg and CPH 20 mg/kg significantly improved locomotion on the 23rd and 43rd days [two-way ANOVA: F(15,90) = 644.72, p < 0.001]. CPH 20 mg/kg was more effective than CPH 10 mg/kg in improving locomotor activity on the 43rd day than CPH 10 mg/kg (Fig. 2e).

### Improved motor coordination after chronic administration with chrysophanol

The beam crossing task was used to assess rats’ motor coordination abilities. The task was completed on the 1st, 13th, 23rd, and 43rd days. There were no significant changes between treatment groups on the first day. On the 13th day, chronic PPA-treated rats experienced considerably more slips than the vehicle, sham, and CPH 20 perse treated groups. Prolonged oral treatment with CPH 10 mg/kg and CPH 20 mg/kg on days 23 and 43 significantly reduced the number of slips in a dose-dependent manner compared to the PPA treatment group [two-way ANOVA: F(15,90) = 35.18, p < 0.001]. CPH 20 mg/kg was substantially more effective than CPH 10 mg/kg in reducing slip count and enhancing beam efficiency on the 43rd day (Fig. 2f).

### Reduced depression-like behavior after chronic administration with chrysophanol

The forced swim test was used to assess rats’ depressive-like behaviour. The immobility time was recorded on the 1st, 13th, 23rd, and 43rd days. There was no significant difference between treatment groups on the first day. PPA-injected rats show longer immobility time significantly on the 13th day of the protocol schedule compared to the vehicle, sham, and CPH 20 perse treatment groups. Long-term oral administration of CPH 10 mg/kg and CPH 20 mg/kg to rats on the 23rd and 43rd days significantly reduces immobility time in a dose-dependent manner when compared to PPA-treated rats [two-way ANOVA: F(15,90) = 910.07, p < 0.001]. On the other hand, CPH 20 mg/kg was found to be more effective than CPH 10 mg/kg in considerably reducing immobility time and restoring depressive-like behaviour on the 43rd day (Fig. 2g).

### Effect of chrysophanol on neurochemical alterations in ICV-PPA induced experimental model of autism in adult rats

#### Decreased PI3K level after chronic administration with chrysophanol

The PI3K protein level was evaluated in rat brain homogenate and CSF samples at the end of the experimental protocol. When compared to the vehicle, sham, and CPH 20 perse treated groups, PPA-treated rats have a significant increase in PI3K protein levels in rat brain homogenate and CSF samples. Long-term oral treatment of CPH at doses of 10 mg/kg and 20 mg/kg for 44 days consistently lower PI3K levels as compared to PPA-treated rats. CPH 20 mg/kg treatment group was shown to be more efficient than CPH 10 mg/kg treatment group in reducing PI3K levels in rat brain homogenate [one-way ANOVA: F(5,25) = 1.136, p < 0.001] and CSF samples [one-way ANOVA: F(5,25) = 0.256, p < 0.001]. (Table 1a.)

#### Decreased AKT level after chronic administration with chrysophanol

The AKT level was determined using an ELISA kit in rat brain homogenate and CSF samples. Chronic PPA injection significantly elevated AKT levels in rat brain homogenate and CSF when compared to the vehicle, sham, and CPH 20 perse treatment groups. Prolonged oral CPH 10 and 20 mg/kg therapy significantly lowered AKT levels in brain homogenate and CSF compared to PPA-injected groups. Furthermore, CPH 20 mg/kg is shown to be more efficient than CPH 10 mg/kg in reducing AKT levels in brain homogenate [one-way ANOVA: F(5,25) = 1.209, p < 0.001] and CSF samples [one-way ANOVA: F(5,25) = 0.151, p < 0.001] (Table 1b).
Table 1 Effect of chrysophanol on PI3K, AKT, mTOR and myelin basic protein level in ICV-PPA induced experimental model of autism in adult rats

| S. no. | Groups      | PI3K Brain homogenate (pg/g protein) | CSF (ng/ml) | AKT Brain homogenate (pg/g protein) | CSF (ng/ml) | mTOR Brain homogenate (ng/g protein) | CSF (ng/ml) | Myelin basic protein Brain homogenate (μg/mg protein) | CSF (ng/ml) |
|--------|-------------|-------------------------------------|-------------|-------------------------------------|-------------|--------------------------------------|-------------|-----------------------------------------------|-------------|
| 1.     | Vehicle control | 7.133 ± 0.142                     | 2.312 ± 0.092 | 3.682 ± 0.103                  | 0.470 ± 0.031 | 1.332 ± 0.081                  | 7.485 ± 0.083 | 110.3 ± 1.972                                      |
| 2.     | Sham control  | 7.123 ± 0.116                     | 2.277 ± 0.105 | 3.743 ± 0.095                  | 0.465 ± 0.029 | 1.308 ± 0.077                  | 7.415 ± 0.129 | 110.1 ± 2.133                                     |
| 3.     | CPH20 perse  | 7.070 ± 0.130                     | 2.298 ± 0.097 | 3.822 ± 0.076                  | 0.460 ± 0.036 | 1.303 ± 0.050                  | 7.422 ± 0.084 | 110.7 ± 1.526                                     |
| 4.     | PPA          | 28.04 ± 0.158*                    | 4.485 ± 0.091* | 19.06 ± 0.197*                | 1.560 ± 0.020* | 7.435 ± 0.101*                | 11.01 ± 0.069* | 50.58 ± 1.040*                                    |
| 5.     | PPA + CPH10  | 21.77 ± 0.146*                    | 3.802 ± 0.076* | 14.71 ± 0.260*                | 1.178 ± 0.046* | 5.865 ± 0.078*                | 9.818 ± 0.087* | 66.67 ± 1.413*                                    |
| 6.     | PPA + CPH20  | 14.16 ± 0.209**                   | 2.795 ± 0.094** | 9.202 ± 0.209**               | 0.858 ± 0.051** | 3.532 ± 0.094**               | 8.792 ± 0.129** | 76.91 ± 1.634**                                    |

Values expressed as mean ± SD (n = 6 rats per group). *p < 0.001 vs vehicle control, sham control and CPH20 Perse; @ p < 0.001 vs PPA; @@ p < 0.001 vs PPA + CPH10 (one-way ANOVA followed by Tukey’s multiple comparison test)

Decreased mTOR level after chronic administration with chrysophanol

The level of mTOR was determined in rat brain homogenate and CSF samples. Long-term PPA-treated rats have higher mTOR levels in rat brain homogenate and CSF samples than the vehicle, sham, and CPH20 groups. Continuous oral administration of CPH 10 and 20 mg/kg significantly reduces mTOR levels in rat brain homogenate [one-way ANOVA: F(5,25) = 1.212, p < 0.001] and CSF [one-way ANOVA: F(5,25) = 0.551, p < 0.001] compared to PPA treatment groups. CPH 20 mg/kg was found to be more efficient in lowering mTOR levels in brain homogenate and CSF samples (Table 1c).

Restored myelin basic protein level after chronic administration with chrysophanol

The level of myelin basic protein (MBP) was determined in rat brain homogenates using an ELISA kit. PPA-injected rats have significantly lower MBP levels when compared to vehicle, sham, and CPH20 perse treated groups. Long-term oral treatment of CPH 10 and 20 mg/kg causes a significant increase in MBP levels as compared to PPA-injected rats [one-way ANOVA: F(5,25) = 1.687, p < 0.001]. CPH 20 mg/kg was more efficient than CPH 10 mg/kg in restoring MBP levels in rat brain homogenate (Table 1d).

Reduction in caspase-3, Bax, and increased Bcl-2 levels after chronic administration with chrysophanol

Neuronal apoptotic markers such as Caspase-3, Bax, and Bcl-2 were measured in rat brain homogenate and blood plasma. Prolonged PPA exposure resulted in significant elevations in Caspase-3 and Bax protein levels in rat brain homogenate and blood plasma. Furthermore, when compared to the vehicle, sham, and CPH20 perse groups, ICV-PPA treated rats have a significant drop in anti-apoptotic Bcl-2 levels in brain homogenate and blood plasma. Caspase-3 levels in brain homogenate [one-way ANOVA: F(5,25) = 0.210, p < 0.001] and blood plasma [one-way ANOVA: F(5,25) = 1.052, p < 0.001] are significantly reduced by persistent oral CPH therapy at 10 and 20 mg/kg.

Similarly, chronic oral CPH treatment at dosages of 10 mg/kg and 20 mg/kg results in a significant reduction in Bax levels in rat brain homogenate [one-way ANOVA: F(5,25) = 1.213, p < 0.001] and blood plasma samples [one-way ANOVA: F(5,25) = 1.246, p < 0.001] Continuous oral treatment with CPH 10 and 20 mg/kg for 44 days resulted in significantly higher Bcl-2 levels in rat brain homogenate [one-way ANOVA: F(5,25) = 2.193, p < 0.001] and blood plasma [one-way ANOVA: F(5,25) = 3.179, p < 0.001]. In comparison, CPH 20 mg/kg was more effective than CPH 10 mg/kg in lowering apoptotic indicators and restoring anti-apoptotic markers in PPA-induced autistic rats (Table 2).

Restoration of neurotransmitters level after chronic administration with chrysophanol

Neurotransmitters such as serotonin, dopamine, acetylcholine, and glutamate were assessed in rat brain homogenate at the end of the treatment schedule. ICV-PPA injections resulted in a significant decrease in dopamine, serotonin, and acetylcholine levels, as well as an increase in glutamate levels, in rat brain homogenate, as compared to vehicle, sham, and CPH20 perse-treated rats. Long-term oral CPH 10 and 20 mg/kg treatment significantly increases dopamine [one-way ANOVA: F(5,25) = 2.546, p < 0.001], serotonin [one-way ANOVA: F(5,25) = 0.228, p < 0.001], and acetylcholine levels while decreasing glutamate...
levels in comparison to PPA-infused autistic rats [one-way ANOVA: F(5,25) = 0.807, p < 0.001]. Among these, CPH 20 mg/kg was more effective than CPH 10 mg/kg in restoring neurotransmitter levels in rat brain homogenate (Table 3).

**Decreased inflammatory cytokines level after chronic administration with chrysophanol**

Using an ELISA kit, inflammatory cytokines such as TNF and IL-1β were quantified in rat brain homogenate and blood plasma samples. Chronic ICC-PPA treatment results in significant increases in proinflammatory mediators such as TNF and IL-1β1 in rat brain homogenate and blood plasma as compared to the vehicle, sham, and CPH20 per se treatment groups. TNF levels in rat brain homogenate [one-way ANOVA: F(5,25) = 0.357, p < 0.001] and blood plasma [one-way ANOVA: F(5,25) = 1.180, p < 0.001] are significantly reduced by continuous oral administration with CPH 10 and 20 mg/kg.

Similarly, long-term treatment with CPH10 and 20 mg/kg significantly reduced IL-1β levels in rat brain homogenate [one-way ANOVA: F(5,25) = 4.582, p < 0.001] and blood plasma [one-way ANOVA: F(5,25) = 0.782, p < 0.001] compared to PPA treated rats. CPH 20 mg/kg treatment, on the other hand, was found to be more effective in lowering inflammatory cytokines when compared to CPH 10 mg/kg treated rats (Table 4).

**Amelioration of oxidative stress markers level after chronic administration with chrysophanol**

In rat brain homogenate, the levels of oxidative stress markers AchE, LDH, SOD, GSH, nitrite, and MDA were evaluated. Chronic PPA administration rats showed a significant increase in AchE, LDH, nitrite, and MDA levels in rat brain homogenate compared to the vehicle, sham, and CPH 20 per se treatment groups. ICC-PPA injected groups showed a significant reduction in anti-oxidant enzyme levels such as SOD [one-way ANOVA: F(5,25) = 0.968, p < 0.001] and GSH [one-way ANOVA: F(5,25)].

Prolonged oral treatment with CPH 10 and 20 mg/kg for 44 days resulted in significantly lower levels of AchE [one-way ANOVA: F(5,25) = 1.266, p < 0.001], LDH

| Table 2 Effect of chrysophanol on apoptotic markers level in ICV-PPA induced experimental model of autism in adult rats |
|----------------|----------------|----------------|----------------|----------------|
| S. no. | Groups | Caspase-3 Brain homogenate (nM/mg protein) | Blood plasma (ng/ml) | Bax Brain homogenate (ng/mg protein) | Blood plasma (ng/ml) | Bcl-2 Brain homogenate (ng/mg protein) | Blood plasma (ng/ml) |
|-------|-------|----------------|----------------|----------------|----------------|----------------|----------------|
| 1. | Vehicle control | 117.4 ± 0.858 | 1.840 ± 0.049 | 9.167 ± 0.269 | 0.833 ± 0.035 | 34.69 ± 0.553 | 9.208 ± 0.036 |
| 2. | Sham control | 118.5 ± 0.622 | 1.830 ± 0.043 | 9.098 ± 0.595 | 0.846 ± 0.033 | 35.22 ± 0.437 | 9.143 ± 0.043 |
| 3. | CPH20 perse | 117.7 ± 1.156 | 1.858 ± 0.046 | 8.690 ± 0.310 | 0.840 ± 0.035 | 34.78 ± 0.919 | 9.153 ± 0.054 |
| 4. | PPA | 178.4 ± 1.313* | 5.798 ± 0.132 | 15.21 ± 0.359* | 5.223 ± 0.092* | 23.34 ± 0.530* | 1.815 ± 0.056* |
| 5. | PPA + CPH10 | 161.0 ± 1.461@ | 3.248 ± 0.136@ | 13.16 ± 0.387@ | 4.315 ± 0.152@ | 25.80 ± 0.725@ | 4.425 ± 0.203@ |
| 6. | PPA + CPH20 | 147.3 ± 1.022@# | 2.493 ± 0.350@# | 10.92 ± 0.275@# | 2.562 ± 0.301@# | 29.27 ± 1.022@# | 7.173 ± 0.103@# |

| Table 3 Effect of chrysophanol on neurotransmitters level in ICV-PPA induced experimental model of autism in adult rats |
|----------------|----------------|----------------|----------------|----------------|
| S. no. | Groups | Neurotransmitters (Brain homogenate) | Serum (ng/mg protein) | Glutamate (ng/mg protein) | Dopamine (ng/mg protein) | Ach (ng/mg protein) |
|-------|-------|----------------|----------------|----------------|----------------|----------------|
| 1. | Vehicle control | 46.99 ± 0.416 | 119.9 ± 0.945 | 102.2 ± 1.867 | 9.047 ± 0.215 |
| 2. | Sham control | 47.22 ± 0.519 | 120.2 ± 0.518 | 101.8 ± 2.186 | 8.955 ± 0.279 |
| 3. | CPH20 perse | 48.09 ± 0.517 | 119.5 ± 0.809 | 102.8 ± 1.852 | 8.855 ± 0.189 |
| 4. | PPA | 17.34 ± 0.648* | 328.4 ± 1.781* | 38.15 ± 1.265* | 2.392 ± 0.104* |
| 5. | PPA + CPH10 | 24.05 ± 0.457@ | 234.4 ± 1.791@ | 54.84 ± 0.842@ | 4.278 ± 0.057@ |
| 6. | PPA + CPH20 | 31.06 ± 0.407@# | 198.6 ± 1.690@# | 61.91 ± 1.163@# | 5.872 ± 0.191@# |

Values expressed as mean ± SD (n = 6 rats per group). *p < 0.001 vs vehicle control, sham control and CPH20 Perse; @ p < 0.001 vs PPA; @# p < 0.001 vs PPA + CPH10 (one-way ANOVA followed by Tukey’s multiple comparison test).
Table 4  Effect of chrysophanol on inflammatory cytokines level in ICV-PPA induced experimental model of autism in adult rats

| S. no. | Groups          | TNF-α (Brain homogenate (pg/mg protein)) | IL-1β (Blood plasma (pg/ml)) |
|--------|-----------------|----------------------------------------|-------------------------------|
| 1.     | Vehicle control | 37.84 ± 0.838                          | 18.69 ± 0.621                |
| 2.     | Sham control    | 37.05 ± 0.548                          | 18.75 ± 0.627                |
| 3.     | CPH20 perse     | 36.90 ± 0.267                          | 18.72 ± 0.702                |
| 4.     | PPA             | 80.45 ± 1.342                          | 34.89 ± 0.478*               |
| 5.     | PPA + CPH10     | 71.40 ± 1.370*                         | 28.84 ± 0.668*               |
| 6.     | PPA + CPH20     | 57.61 ± 1.850**                        | 23.64 ± 0.440**              |

Values expressed as mean ± SD (n=6 rats per group). *p < 0.001 v/s vehicle control, sham control and CPH20Perse; @ p < 0.001 v/s PPA; @@ p < 0.001 v/s PPA + CPH10 (one-way ANOVA followed by Tukey’s multiple comparison test)

Table 5  Effect of chrysophanol on oxidative stress markers level in ICV-PPA induced experimental model of autism in adult rats

| S. no. | Groups          | Oxidative stress markers (Brain homogenate) |
|--------|-----------------|---------------------------------------------|
|        |                 | AchE (μM/mg protein) | LDH (Unit/mg protein) | SOD (μM/mg protein) | GSH (μM/mg protein) | Nitrite (μM/mg protein) | MDA (nM/mg protein) |
| 1.     | Vehicle control | 23.86 ± 0.971        | 130.1 ± 1.206        | 492.3 ± 2.583       | 38.27 ± 0.790       | 6.89 ± 0.261           | 36.91 ± 0.315       |
| 2.     | Sham control    | 24.24 ± 0.790        | 125.4 ± 0.545        | 491.9 ± 3.425       | 38.63 ± 0.886       | 7.11 ± 0.220           | 37.67 ± 0.622       |
| 3.     | CPH20 perse     | 23.90 ± 0.886        | 126.6 ± 0.628        | 493.1 ± 3.004       | 39.10 ± 0.421       | 6.91 ± 0.181           | 37.11 ± 1.079       |
| 4.     | PPA             | 60.25 ± 1.214*       | 428.7 ± 1.835*       | 347.8 ± 1.806       | 10.95 ± 0.422*      | 13.41 ± 0.458*        | 80.00 ± 0.880*      |
| 5.     | PPA + CPH10     | 45.41 ± 0.874*       | 318.1 ± 1.057*       | 359.8 ± 0.914*      | 16.88 ± 0.732*      | 10.82 ± 0.450*        | 71.19 ± 0.824*      |
| 6.     | PPA + CPH20     | 36.97 ± 1.346**      | 289.8 ± 2.058**      | 393.2 ± 2.183**     | 24.76 ± 0.769**     | 8.85 ± 0.234**        | 63.37 ± 0.881**     |

Values expressed as mean ± SD (n=6 rats per group). *p < 0.001 v/s vehicle control, sham control and CPH20Perse; @ p < 0.001 v/s PPA; @@ p < 0.001 v/s PPA + CPH10 (one-way ANOVA followed by Tukey’s multiple comparison test)

Effect of chrysophanol in the restoration of gross pathological alterations in ICV-PPA induced experimental model of autism in adult rats

Restoration of whole-brain alterations after chronic administration with chrysophanol

The normal, vehicle, and CPH 20 perse treated groups all showed normal brain size and morphology. In comparison to the vehicle, sham, and CPH 20 perse treatment groups, the ICV-PPA-treated rat brains had a disrupted clotted outermost layer with rupture meninges. Prolonged oral administration of CPH at 10 mg/kg and 20 mg/kg doses repaired the morphological changes and aided the rat brain’s recovery from subsequent injury. Similarly, animals given CPH 20 mg/kg showed considerable recovery in the damaged area of the brain as well as recovery of brain injury when compared to rats given CPH 10 mg/kg (Fig. 3a).

Reduction of pathological changes in brain sections after chronic administration with chrysophanol

Brain sections of rats treated with vehicle, sham, and CPH 20 mg/kg perse were structurally intact and undamaged, with clearly visible basal ganglia, cortex, and hippocampal tissue. The brain sections of the ICV-PPA treated rats showed cortical and hippocampus shrinkage, as well as atrophy in subcortical areas such as the medial thalamus, putamen, caudate nucleus, and internal medullary lamina, as compared to the vehicle, sham, and CPH 20 perse, treated rats. The pathological abnormalities in rat brain slices were reversed by long-term oral treatment of CPH 10 and 20 mg/kg (Fig. 3b).
Reduction in demyelination volume after chronic administration with chrysophanol

The normal, vehicle, and CPH20 perse treated groups all demonstrated no significant change in demyelination volume. However, when compared to the normal, vehicle, and CPH20 treatment groups, long-term administration of the neurotoxin PPA for 11 days significantly increased the area of demyelination. Long-term oral CPH administration at dosages of 10 mg/kg and 20 mg/kg significantly reduced demyelination volume in autistic-like rats compared to PPA-treated autistic-like rats. As a result, CPH 20 mg/kg had a dose-dependent effect on demyelination volume reduction when compared to CPH 10 mg/kg treated rats [one-way ANOVA: F(5,25) = 0.241, p > 0.001] (Fig. 4).

Fig. 3 Effect of chrysophanol in the restoration of gross pathological alterations of whole rat brain and brain sections in ICV-PPA induced experimental model of autism in adult rats. (a) Whole rat brain. (a) Vehicle control (b) Sham control (c) CPH20 perse (d) PPA (e) PPA + CPH10 (f) PPA + CPH20 (Scale bar = 2 mm) Note: Yellow circles are pointing to the site of the brain injury. (b) Brain sections. (a) Vehicle control (i) Cerebral cortex (ii) Hippocampus (iii) basal Ganglia (b) Sham control (c) CPH20 perse (d) PPA (e) PPA + CPH10 (f) PPA + CPH20 (Scale bar = 5 mm) Note: Yellow circles are pointing to the injured site.

Fig. 4 Effect of chrysophanol on demyelination volume in ICV-PPA induced experimental model of autism in adult rats. (Values expressed as mean ± SD (n = 6 rats per group). *p < 0.001 vs vehicle control, sham control and CPH-20Perse; @ p < 0.001 vs PPA; @# p < 0.001 vs PPA + CPH10 (one-way ANOVA followed by Tukey’s multiple comparison test)
Discussion

The current study used a PPA-induced experimental adult rat model to evaluate neurobehavioral and neurochemical abnormalities in autistic-like animals. Several investigations have found that ICV-PPA injection plays a significant role in developing autistic-like behaviour in adult rats (Khera et al. 2022a, 2022b; Rahi et al. 2021; Tiwari et al. 2021; Sharma et al. 2019). PPA-exposed rats exhibit behavioural and neuropathological abnormalities that are comparable to those seen in ASD patients, including hyperactivity, poor social interaction, stereotypic and repetitive movements, and have been recognized as a viable adult ASD model in rodents (Nemecek and Moore 2020; Chow et al. 2012).

PPA, as a weak organic acid, can passively accumulate within CNS cells, resulting in a fall in intracellular pH, which has several physiological implications (Thomas et al. 2010). PPA exerts a range of physiological effects on the brain, corresponding to the increased locomotion found in experimental rats (Lobzhanidze et al. 2020). As a result, it can alter neurotransmission in brain regions related to locomotor behaviour, such as the hippocampus and prefrontal cortex (Meeking et al. 2020; MacFabe et al. 2007). PPA-treated rat brains had higher amounts of cytokines and oxidative stress markers, as well as other abnormalities associated with ASD (MacFabe et al. 2007; Bhandari and Kuhad 2017).

CPH was investigated to see whether it may protect against the behavioural changes caused by neurotoxin PPA in an experimental model of autism in adult rats. PPA infusion causes ASD-like behavioural and neuroinflammatory responses in adult rats. It is a neurotoxin that alters rat behaviour by impairing learning and memory, disrupting social interactions, and causing anxiety (Shultz et al. 2008; Ku et al. 2016; Wu et al. 2017). Additionally, we measured the protein levels of various cellular and molecular markers, apoptotic markers, neurotransmitters, inflammatory cytokines, and oxidative stress parameters in rat brain homogenate, blood plasma, and cerebrospinal fluid (CSF) samples. Long-term oral treatment of CPH at two different doses showed a neuroprotective effect against neurobehavioral and neurochemical changes in the ICV-PPA induced experimental model of autism in adult rats.

Bodyweight measurements taken on several days revealed that rats significantly decreased bodyweight after 11 days of ICV-PPA infusions. Prior research has shown that PPA, a short-chain fatty acid, influences weight loss in both animal and human populations due to altered fatty acid metabolism and increased gluconeogenesis after entering the citric acid cycle (Nankova et al. 2014). (Choi et al. 2018). In the current investigation, this weight loss increased in a dose-dependent manner after CPH administration. The brain-body weight ratio was also calculated by dividing the fresh brain weight by the bodyweight at the end of the experimental treatment schedule. The brain-body weight ratio was significantly lower in ICV-PPA-treated autistic rats, although it increased dose-dependently with CPH therapy. During the forced swim test, the immobility time was used to assess depressive-like behaviour in ICV-PPA induced experimental model of autism in adult rats. A previous study found that PPA therapy had an increased depressed effect (Bhandari and Kuhad 2017). The current study’s findings revealed an increase in immobility time after PPA injection that was mitigated by continuous CPH treatment.

The PI3K/Akt signaling pathway has an important role in regulating neuronal metabolism, neuoinflammation, cell development and survival (Sharma et al. 2021; Lin et al. 2020). Moreover, PI3K/Akt/ mTOR signaling pathway is demonstrated to be correlated to neuroprotection and is over-expressed at the occurrence of neurodegeneration (Fakhri et al. 2021). Various studies have implied that PI3K is involved in synaptic plasticity, learning, memory, and major depression (Budni et al. 2012; Horwood et al. 2006). Formation of lipid products followed by PI3K activation, act as second messengers by employing proteins, such as protein kinase B (PKB/Akt) that results in the activation of its downstream kinases and consequently increasing mTOR phosphorylation (Hoeffer and Klann 2010). It has been demonstrated that Akt is a central regulator of disease progression due to its vast signaling in the ASD brain (Gazestani et al. 2019).

Activation of mTOR signaling stimulates messenger RNA (mRNA) translation and protein synthesis by activating p70S6 kinase (p70S6K). This produces quick and sustained elevation of synapse-associated proteins, including postsynaptic density-95 protein (PSD95) responsible for scaffolding, organization of receptors, such as α-aminobutyric acid (GABA) receptors, modulation of the voltage-gated ion channels expression and alterations in the neurotransmitters expression levels (Hoeffer and Klann 2010; Niere and Raab-Graham 2017). The various GPCRs found to activate mTOR in neurons includes the glutamate metabotropic mGlu1/5 receptors (Banko et al. 2006; Ronesi and Huber 2008), the μ-opioid receptor (Polakiewicz et al. 1998), the dopaminergic D1 (Santini et al. 2009) and D3 receptors (Salles et al. 2013), the amino acid/glutamate T1R1-T1R3 receptors (Wusson et al. 2012), the serotonin 5-HT6 receptor (Meffre et al. 2012), the GABA_A receptors (Workman et al. 2013) and the cannabin (CB1) receptor (Meffre et al. 2012). The CB1 receptors activates mTOR indirectly which depends on an inhibition of GABA release from inhibitory GABAergic interneurons (Pugier et al. 2009). This leads to an enhanced activity of the excitatory networks including glutamate NMDA receptor activation and, consequently,
mTOR activation (Puighermanal et al. 2009). This causes hyperexcitability in ASD-like conditions. It has been previously reported that the upregulation of mTOR increases the levels of pro-inflammatory cytokines and decreases the anti-inflammatory cytokines production (Jadaun et al. 2022a, 2022b). Moreover, pro-inflammatory cytokine increases glutamate release from microglial cells via upregulation of glutaminase, stimulating AMPA receptor expression, and inducing GABA receptor endocytosis, resulting in changes in the neuronal excitability (Sanz and Garcia-Gimeno 2020). Previous research has linked the PI3K/AKT/GSK3/mTOR/BDNF pathway to the development of mood-related disorders and has also been linked to the adaptive stress response (Lu et al. 2015). Upregulated PI3K-AKT/mTOR signaling pathway activity in neurons is linked with stereotypically autistic behaviors, including memory and learning changes, serotonergic impairment, epilepsy, and changes to both structural and synaptic plasticity (Costa-Mattioli and Monteggia 2013; Hutsler and Zhang 2010). Numerous research has revealed an association between PI3K/AKT/mTOR and depression and anxiety (Leibrock et al. 2013; Moretti et al. 2014). In a chronic stress model of depression in mice, CPH treatment promotes antidepressant efficacy by blocking the mTOR signalling pathway (Zhang et al. 2016). All of these data confirmed our study’s findings that CPH reduced depression symptoms in the PPA-induced experimental model of autism in adult rats.

Additionally, the hyperactivity and repetitive behaviours associated with autistic individuals have been highlighted as primary symptoms (Kong et al. 2021). As a result, PPA and other short-chain fatty acids increase glial and intracellular neuronal acidification and calcium levels, affecting neurotransmitter release, including serotonin, dopamine, glutamate, and norepinephrine (Daghestani et al. 2017; Thomas et al. 2012). PPA has also been shown to enhance glutamatergic transmission, which causes excitability in brain areas related with locomotor activity. Our locomotor activity results showed increased locomotion after ICV-PPA injection, which is consistent with the findings of our previously reported studies (Tiwari et al. 2021; Sharma et al. 2019). There was a significant and dose-dependent improvement in hyperactive and repetitive behaviour after CPH treatment at doses of 10 mg/kg and 20 mg/kg. The beam crossing task assessed balance and motor coordination by counting the number of slips while moving across a wooden beam. Our results show that PPA autistic rats had a higher number of slips, indicating poor motor coordination, which was reduced in a dose-dependent way after CPH treatment.

Autistic children are reported to have poor spatial memory, cognitive impairments, and intellectual deficiencies (Zhang et al. 2020). The Morris water maze was used to test rats’ long-term memory and spatial learning abilities. Mepham et al. found that intracerebroventricular PPA injection decreased spatial cognition in adult rats (Mephem et al., 2019). Our results demonstrated that rats treated with PPA had severe memory loss as a result of increased ELT and decreased TSTQ. Additionally, CPH treatment reduces the escape latency time (ELT), even though increased TSTQ in MWM indicates enhanced spatial memory.

In order to investigate a cellular signalling mechanism, we also examined the effect of CPH on the PI3K/AKT/mTOR protein levels. Upregulation of PI3K/AKT/mTOR has been linked to the onset and progression of neurological dysfunctions (Bozdaği et al. 2013; Chen et al. 2014). Recently, this signaling pathway overactivation has been linked to pathological processes that may be responsible for autism (Kwon et al. 2006). In comparison, inhibiting the PI3K/AKT/mTOR signalling pathway resulted in a neuroprotective effect that was employed as a diagnostic marker in autistic patients (Yeung et al. 2017). As a result, we found elevated PI3K/AKT/mTOR levels in the CSF and brain homogenate of ICV-PPA induced experimental model of autism. However, CPH therapy decreases the PI3K/AKT/mTOR protein levels, which protects against autism by lowering apoptosis, neuroinflammation, oxidative stress in CSF and rat brain homogenate.

Researchers used new techniques to detect abnormalities in the white matter region of the brain that is connected with autistic dysfunction. A previous study revealed that MBP levels were lower in the brains of autistic patients (Gonzalez-Gronow et al. 2015). The current study found decreased MBP levels in ICV-PPA rats’ brain homogenate. Furthermore, CPH administration restored MBP levels in autistic rats’ brain homogenates. Apoptotic cell death impairs brain maturation and is thought to be a risk factor for the development of autism (Eftekharian et al. 2019). PPA exposure elevated the levels of apoptotic indicators such as Bax and Caspase 3, while decreasing the levels of anti-apoptotic marker Bcl-2. Long-term CPH therapy has been demonstrated to protect cells against cell death by decreasing Bax and Caspase-3 levels while increasing Bcl-2 levels.

The neurochemical analysis in our study provides a clear indication of CPH neuroprotective potential. Neurotransmitters, which regulate memory, emotion, and behaviour, must balance normal function and neuronal development. Neurotransmitter disruption was identified to be one of the primary causes of the onset of behavioural traits. One of the most prominent aspects of autism is neurotransmitter imbalance (Kuo and Liu 2018). The most investigated neurotransmitters in autism include serotonin, dopamine, glutamate, and Ach. As a result of serotonin’s role in the brain’s development, autistic individuals exhibit socially impaired, repetitive, and depressive behaviour (Kane et al. 2012; Amodeo et al. 2021).
Furthermore, a decrease in dopamine, Ach, and an increase in glutamate were linked to autistic behaviour (Drenthen et al. 2016; Karvat and Kimchi 2014; DiCarlo et al. 2019). Increased glutamate levels activate microglia and promote neuroinflammation, whereas decreasing dopamine and acetylcholine levels influence neuronal excitability and cause mood abnormalities (Acharjee et al. 2018). Our results show that repeated ICV-PPA injections considerably affect the amount of neurotransmitters in rat brain homogenates. Dopamine, serotonin, and acetylcholine levels in ICV-PPA-treated rats decreased, whereas glutamate levels significantly increased, indicating neuronal excitotoxicity. CPH treatment restores neurotransmitter levels in a dose-dependent manner and improves autistic-like behaviour.

TNF and IL-1 are significant mediators of oxidative stress, and neuroinflammation are implicated in neurodegenerative diseases (Saghatzadeh et al. 2019; Mirza and Sharma 2019). Clinical studies on autistic children clearly showed higher inflammatory cytokine levels in CSF, resulting in immunological response and brain damage (Chez et al. 2007). According to our findings, PPA infusion elevated inflammatory cytokines such as TNF and IL-1β in blood plasma and brain homogenate. CPH treatment significantly decreases inflammatory cytokine levels in blood plasma and brain homogenate, resulting in anti-inflammatory actions. Previous research has identified elevated oxidative stress as one of the pathogenic characteristics of autism (Morimoto et al. 2020; Abruzzo et al. 2019; El-Ansary et al. 2012). We measured oxidative stress markers in the brain homogenates of PPA and CPH-treated rats to characterize the severity of the disease and the preventive effects of CPH against oxidative stress. Our data clearly reveal an increase in AchE, LDH, nitrite, and MDA, as well as a decrease in antioxidants, particularly SOD and GSH, in an ICV-PPA induced experimental model of autism in adults rats. PPA-induced autistic rats treated with CPH showed significantly reduced levels of oxidative stress markers, and showing antioxidant properties.

This study looks at the morphological structure of the brain, whole-brain sections, and demyelination volume. Previous research has indicated that the hippocampus is relatively sensitive to PPA exposure. The gross pathological and morphological findings show that PPA-treated rats’ brains differ in size and shape (Rahi et al. 2021). Prolonged CPH administration improved morphological abnormalities in ICV-PPA-treated autistic rats, such as damaged meninges and constricted prefrontal cortex. Coronal sections of ICV-PPA-treated rats exhibited malformed basal ganglia, a defragmented hippocampal area, and degraded white matter. Furthermore, the measurement of demyelination volume in rat brains revealed a significant decrease in white matter volume following PPA injections (Rahi et al. 2021). Continuous CPH therapy was found to lessen the severity of pathological and morphological changes. Long-term treatment of CPH10 mg/kg and CPH20 mg/kg to autistic rats recovered abnormalities in brain sections and enabled remyelination of damaged areas as compared to ICV-PPA treated autistic rats.

Our research primarily focuses on CPH’s neuroprotective potential by decreasing the PI3K/AKT/mTOR protein levels, alleviating behavioural, neurochemical, morphological, and gross pathological abnormalities in the ICV-PPA induced experimental model of autism in adult rats. Concurrent studies, such as Western Blot and immunohistochemistry, are also required to provide molecular support for this hypothesis. Despite these limitations, CPH’s neuroprotective potential in resolving or downregulating aberrant PI3K/AKT/mTOR signalling pathways in the CNS appears promising.

**Conclusion**

Based on the findings, we conclude that PPA injection causes considerable changes, including irregular neural cell structure, autism-like neurobehaviors, and neurochemical abnormalities. To date, there have been no pre-clinical investigations on the neuroprotective effect of CPH via PI3K/AKT/mTOR signalling downregulation in an ICV-PPA generated experimental model of autism in adult rats. It has been observed that PPA can cause autistic-like behaviour in experimental rats. Our findings suggest that CPH substantially improves social interaction, learning, and memory deficiencies in PPA-exposed rats. CPH significantly lowered oxidative stress, neuroinflammation, and apoptotic cell death in autistic rats by reducing the PI3K/AKT/mTOR protein levels in brain homogenate, blood plasma and CSF samples. Furthermore, the recovery of gross pathological defects in the whole brain and brain sections demonstrates CPH’s potential to protect against PPA-induced neurological impairments. However, additional genetic study and immunohistochemical investigation are necessary to explain the underlying pathways governing such interactions. Indeed, PI3K/AKT/mTOR can be used as a therapeutic target in combination with other standard pharmacological interventions.

**Abbreviations**

CNS: Central nervous system; mTOR: Mammalian target of rapamycin; PI3K: Phosphoinositide 3-kinase; CPH: Chrysophanol; AKT: Protein kinase B (PKB); ASD: Autism spectrum disorder; AD: Alzheimer’s disease; ASD: Autism spectrum disorder; BBB: Blood brain barrier; DA: Dopamine; ICV: Intracerebroventricular; PD: Parkinson’s disease; PPA: Propionic acid; SOD: Superoxide dismutase; MDA: Malondialdehyde; TNF: Tumor necrosis factor; GSH: Glutathione; LDH: Lactate dehydrogenase; IL-1β: Interleukin-1β; HPLC: High performance liquid chromatography; AchE: Acetylcholinesterase; Ach: Acetylcholine; TSTQ: Time spent in target quadrant; ELT: Escape latency time; MWM: Morris water maze; BCT: Beam crossing task; FST: Forced swim test; MBP: Myelin basic protein; AD: Alzheimer’s disease
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11011-022-01026-0.

Acknowledgments The authors express their gratitude to Chairman, Mr. Parveen Garg, and Director, Dr. G.D.Gupta, ISF College of Pharmacy, Moga (Punjab), India, for their excellent vision and support.

Authors contribution Ms. Aarti Sharma (First Author).
M. Pharm, Neuropharmacology Division, Department of Pharmacology.
ISF College of Pharmacy, Moga, Punjab, India.
Contribution: Thesis research work, Performed experimental animal studies.

Ms. Sonalika Bhalla (Second author).
M. Pharm, Neuropharmacology Division, Department of Pharmacology.
ISF College of Pharmacy, Moga, Punjab, India.
Contribution: Revision of entire research manuscript, editing.

Dr. Sidharth Mehan (Corresponding Author and Third Author).
PhD, M. Pharm, Neuropharmacology Division, Department of Pharmacology.
ISF College of Pharmacy, Moga, Punjab, India.
Contribution: Original research hypothesis, guide, and compilation of all manuscript data.

Contact: +91–8,059,889,909, +91–9,461,322,911.
Note: All authors approved the final version of this study.

Funding This work was supported by institutional grants from the Institutional Animal Ethics Committee (IAEC) with registration no. 816/PO/ReBiBs/S/04/CPCSEA as protocol no. IAEC/CPCSEA/Meeting No: 27/2020/Protocol No. 454approved by RAB Committee, ISFCP, Moga, Punjab, India.

Data availability All data generated or analyzed during this study are included in this article. There are no separate or additional files.

Declarations

Disclosure of potential conflicts of interest The authors declare that they have no competing interests.

Ethical approval All applicable institutional guidelines for the care and use of animals were followed.

References

Abd-Elrahman KS, Ferguson SS (2019) Modulation of mTOR and CREB pathways following mGluR5 blockade contribute to improved Huntington’s pathology in zQ 175 mice. Mol Brain 12(1):1–9
Abruzzo PM, Matté A, Bolotta A, Federti E, Ghezzo A, Guarneri T et al (2019) Plasma peroxiredoxin changes and inflammatory cytokines support the involvement of neuro-inflammation and oxidative stress in autism Spectrum disorder. J Transl Med 17(1):1–12
Acharjee S, Verbeek M, Gomez CD, Bishk K, Lee B, Benoit L, Sharkey KA, Benediktsson A, Tremblay ME, Pittman QJ (2018) Reduced microglial activity and enhanced glutamate transmission in the basolateral amygdala in early CNS autoimmunity. J Neurosci 38(42):9019–9033. https://doi.org/10.1523/JNEUROSCI.0398-18.2018 Erratum in: J Neurosci. 2019 May 6
Amodeo DA, Oliver B, Pahua A, Hitchcock K, Bykowski A, Tice D et al (2021) Serotonin 6 receptor blockade reduces repetitive behavior in the BTBR mouse model of autism spectrum disorder. Pharmacol Biochem Behav 200:173076
Banko JL, Hou L, Poulin F, Sonenberg N, Klann E (2006) Regulation of eukaryotic initiation factor 4E by converging signaling pathways during metabotropic glutamate receptor-dependent long-term depression. J Neurosci 26(8):2167–2173. https://doi.org/10.1523/JNEUROSCI.5196-05.2006
Bhandari R, Kuhad A (2017) Resveratrol suppresses neuroinflammation in the experimental paradigm of autism spectrum disorders. Neurochem Int 103:8–23. https://doi.org/10.1016/j.neuint.2016.12.012
Bozdagi O, Tavassoli T, Buxbaum JD (2013) Insulin-like growth factor-1 rescues synaptic and motor deficits in a mouse model of autism and developmental delay. Mol Autism 4(1):9. https://doi.org/10.1186/2040-2392-4-9
Brandt C, Hillmann P, Noack A, Römermann K, Öhler LA, Rageot D, Beaufils F, Melone A, Sele AM, Wymann MP, Fabbro D (2018) The novel, catalytic mTORC1 inhibitor PQR620 and the PI3K/mTORC1/2 inhibitor PQR530 effectively cross the blood-brain barrier and increase seizure threshold in a mouse model of chronic epilepsy. Neuropharmacology. 15(140):107–120
Budni J, Lobato KR, Binfaré RW, Freitas AE, Costa AP, Martín-de-Savedra MD, Leal RB, Lopez MG, Rodrigues AL (2012) Involvement of PI3K, GSK-3β and PPARγ in the antidepressant-like effect of folic acid in the forced swimming test in mice. J Psychopharmacol 26(5):714–723. https://doi.org/10.1177/0269881111424456
Chae U, Min JS, Leem HH, Lee HS, Lee HJ, Lee SR, Lee DS (2017) Chrysophanol suppressed glutamate-induced hippocampal neuronal cell death via regulation of dynamin-related protein 1-dependent mitochondrial fission. Pharmacology. 100(3–4):153–160. https://doi.org/10.1159/000477814
Charan J, Kantharia ND (2013 Oct) How to calculate sample size in animal studies? J Pharmacol Pharmacother 4(4):303–306. https://doi.org/10.4103/0976-500X.119726
Chen J, Alberts I, Li X (2014) Dysregulation of the IGF-I/PI3K/AKT/mTOR signaling pathway in autism spectrum disorders. Int J Dev Neurosci 35:35–41. https://doi.org/10.1016/j.jidneu.2014.03.006
Chen Y, Zheng X, Wang Y, Song J (2019) Effect of PI3K/Akt/mTOR signaling pathway on JNK3 in parkinsonian rats. Exp Ther Med 17(3):1771–1775
Chez MG, Dowling T, Patel PB, Khanna P, Kominsky M (2007) Elevation of tumor necrosis factor-alpha in cerebrospinal fluid of autistic children. Pediatr Neurol 36(6):361–365
Choi J, Lee S, Won J, Jin Y, Hong Y, Hur TY et al (2018) Pathophysiological and neurobehavioral characteristics of a propionic acid-mediated autism-like rat model. PLoS One 13(2):e0192925
Chow ML, Pramparo T, Winn ME, Barnes CC, Li HR, Weiss L, Fan JB, Murray S, April C, Belinson H, Fu XD, Wynshaw-Boris A, Schork NJ, Courchesne E (2012) Age-dependent brain gene expression and copy number anomalies in autism suggest distinct pathological processes at young versus mature ages. PLoS Genet 8(3):e1002592. https://doi.org/10.1371/journal.pgen.1002592
Chu X, Zhou S, Sun R, Wang L, Xing C, Liang R, Kong Q (2018) Chrysophanol relieves cognition deficits and neuronal loss in a mouse model of chronic epilepsy. Neurochem Res 43(4):972–983. https://doi.org/10.1007/s11064-018-2503-1
Costa-Mattioli M, Monteggia LM (2013) mTOR complexes in neu- rodevelopmental and neuropsychiatric disorders. Nat Neurosci 16(11):1537–1543
Kim YC, Guan KL (2015) mTOR: a pharmacologic target for autism. PLoS One 7(11):e48975

Kang DW, Ilhan ZE, Isern NG, Hoyt DW, Howsmon DP, Shaiffer M, Lozupone CA, Hahn J, Adams JB, Krajmalnik-Brown R (2018) Differences in fecal microbial metabolites and microbiota of children with autism spectrum disorders. Anaerobe. 49:121–131. https://doi.org/10.1016/j.anaerobe.2017.12.007

Karvat G, Kimichi T (2014) Acetylcysteine elevation relieves cognitive rigidity and social deficiency in a mouse model of autism. Neuropsychopharmacology 39(4):831–840

Kassai H, Sugaya Y, Noda S, Nakao K, Maeda T, Kano M, Aiba A (2014) mTOR pathway. Phytother Res 25(6):833–837. https://doi.org/10.1002/ptr.3323

Lee JM, Kyecno S, Kim E, Cheon KA (2016) Abnormalities of inter-and intra-hemispheric functional connectivity in autism spectrum disorders: a study using the autism brain imaging data exchange database. Front Neurosci 10:191. https://doi.org/10.3389/fnins.2016.00191

Leibrock C, Ackermann TF, Hierlemmeier M, Lang F, Borgwardt S, Lang UE (2013) Akt2 deficiency is associated with anxiety and depressive behavior in mice. Cell Physiol Biochem 32(3):766–777. https://doi.org/10.1159/000354478

Li G, Lu X, Zhang S, Zhou Q, Zhang L (2015) mTOR and Erk1/2 signaling in the cerebrospinal fluid-contacting nucleus is involved in neuropathic pain. Neurochem Res 40(3):1053–1062. https://doi.org/10.1007/s11064-015-1564-7

Li N, Lee B, Liu RJ, Banar M, Dwyer JM, Iwata M, Li XY, Aghajanian G, Duman RS (2010) mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science. 329(5994):959–964. https://doi.org/10.1126/science.1190287

Lian Y, Xia X, Zhao H, Zhu Y (2017) The potential of chrysophanol in protecting against high fat-induced cardiac injury through Nrf2-regulated anti-inflammation, anti-oxidant, and anti-fibrosis in Nrf2 knockout mice. Biomed Pharmacother 93:1175–1189. https://doi.org/10.1016/j.biopha.2017.05.148

Lin JY, Kuo WW, Baskaran R, Kuo CH, Chen YA, Chen WS, Ho TJ, Day CH, Mahalakshmi B, Huang CY (2020) Swimming exercise stimulates IGF1r/PI3K/Akt and AMPK/SIRT1/PGC1α survival signaling to suppress apoptosis and inflammation in aging hippocampus. Aging (Albany NY) 12(8):6852–6864. https://doi.org/10.18632/aging.103046 Erratum in: Aging (Albany NY). 2020 Aug 30;12(16):16663–16664

Lobzhanidze G, Iaparidze N, Lordkipanidze T, Rzayev F, MacFabe D, Zhvania M (2020) Behavioural and brain ultrastructural changes following the systemic administration of propionic acid in adolescent male rats. Further development of a rodent model of autism. Int J Dev Neurosci 80(2):139–156. https://doi.org/10.1016/j.ijdevneu.2010.01.001

Lu CC, Yang JS, Huang AC, Hsia TC, Chou ST, Kuo CL, Lu HF, Lee TH, Wood WG, Chung JG (2010a) Chrysophanol induces necrosis through the production of ROS and alteration of ATP levels in J5 human liver cancer cells. Mol Nutr Food Res 54(7):967–976. https://doi.org/10.1002/mnfr.2009000265

Lu CC, Yang JS, Huang AC, Hsia TC, Chou ST, Kuo CL, Lu HF, Lee TH, Wood WG, Chung JG (2010b) Chrysophanol induces necrosis through the production of ROS and alteration of ATP levels in J5 human liver cancer cells. Mol Nutr Food Res 54(7):967–976. https://doi.org/10.1002/mnfr.2009000265

Luo L, Li K, Mao Y-H, Qu H, Yao B, Zhong W-W et al (2017) Gold-chrysophanol nanoparticles suppress human prostate cancer progression through inactivating AKT expression and inducing apoptosis and ROS generation in vitro and in vivo. Int J Oncol 51(4):1089–1103. https://doi.org/10.3892/ijo.2017.4095

Lu Y, Wang C, Xue Z, Li C, Zhang J, Zhao X et al (2015) PI3K/AKT/mTOR signaling-mediated neurupetide VGF in the Hippocampus of mice is involved in the rapid onset antidepressant-like effects of GLYX-13. Int J Neuropsychopharmacol 18(5). https://doi.org/10.1017/jnp.2014.110

MacFabe DF, Cain DP, Rodriguez-Capote K, Franklin AE, Hoffman JE, Boon F, Taylor AR, Kavaliers M, Ossenkopp KP (2007) Neurobiological effects of intraventricular propionic acid in rats: possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders. Behav Brain Res 176(1):149–169. https://doi.org/10.1016/j.bbr.2006.07.025

Maiti P, Manna I, Ilavazhagan G, Rossignol J, Dunbar GL (2015) Molecular regulation of dendritic spine dynamics and their potential impact on synaptic plasticity and neurological diseases. Neurosci Biobehav Rev 59:208–237. https://doi.org/10.1016/j.neubiorev.2015.09.020

Meeking MM, MacFabe DF, Mepham JR, Foley KA, Tichenoff LJ, Boon FH, Kavaliers M, Ossenkopp KP (2020) Propionic acid
induced behavioural effects of relevance to autism spectrum disorder evaluated in the hole board test with rats. Prog Neuro-Psychopharmacol Biol Psychiatry 97:1097–94. https://doi.org/10.1016/j.pnpbp.2019.109794

Meffre J, Chaumont-Dubel S, Mannoury la Cour C, Loiseau F, Watson DJ, Dekeyne A, Sévén M, Rivet JM, Gaven F, Déléris P, Hervé D, Fone KC, Boackaert J, Millan MJ, Marin P (2012) 5-HT(6) receptor recruitment of mTOR as a mechanism for perturbed cognition in schizophrenia. EMBO Mol Med 4(10):1043–1056. https://doi.org/10.1002/emmm.201204140

Mehan S, Monga V, Rani M, Dudi R, Ghimire K (2018) Neuroprotective effect of solanolex against 3-nitropropionic acid-induced Huntington’s disease-like behavioral, biochemical, and cellular alterations: restoration of coenzyme-Q10-mediated mitochondrial dysfunction. Indian J Pharm 50(6):309–319. https://doi.org/10.4103/ijp.IJP_11_18

Mehan S, Rahi S, Tiwari A, Kapoor T, Rajdev K, Sharma R, Khera H, Kosey S, Kukkar U, Dudi R (2020) Adenylate cyclase activator forskolin alleviates intracerebroventricular propionic acid-induced mitochondrial dysfunction of autistic rats. Neural Regen Res 15(6):1140–1149. https://doi.org/10.4103/1673-5374.270316

Mehan S, Parveen S, Kalra S (2017) Adenyl cyclase activator forskolin protects against Huntington’s disease-like neurodegenerative disorders. Neural Regen Res 12(2):290–300. https://doi.org/10.4103/1673-5374.200812

Mepham JR, Boon FH, Foley KA, Cain DP, MacFabe DF, Ossenkopp KP (2019) Impaired spatial cognition in adult rats treated with multiple Intracerebroventricular (ICV) infusions of the enteric bacterial metabolite, propionic acid, and return to baseline after 1 week of no treatment: a control study of a rodent model of ASD. Neurotox Res 35(4):823–837. https://doi.org/10.1007/s12640-019-0002-z

Mijn E, Upadhayay S, Mehan S (2021) Nrf2/HO-1 signaling activator Acetyl-11-keto-beta Boswellic acid (AKBA)-mediated neuroprotection in methyl mercury-induced experimental model of ALS. Neurochem Res. https://doi.org/10.1007/s11064-021-03366-2

Mirza R, Sharma B (2019) A selective peroxisome proliferator-activated receptor-γ agonist benefitted propionic acid induced autism-like behavioral phenotypes in rats by attenuation of neuroinflammation and oxidative stress. Chem Biol Intecct 311:108578. https://doi.org/10.1016/j.cbi.2019.108578

Moretti M, Budni J, Freitas AE, Rosa PB, Rodrigues AL (2014) Antidepressant-like effect of ascorbic acid is associated with the modulation of mammalian target of rapamycin pathway. J Antidepressant-like effect of ascorbic acid is associated with the modulation of mammalian target of rapamycin pathway. J

Mobarakeh A, Rezaei N (2019) A meta-analysis of pro-inflammatory cytokines in autism spectrum disorders. J Biomed Sci. 26(1):1–19. https://doi.org/10.1186/s12920-019-0273-5

Nancakova BB, Agarwal R, MacFabe DF, Rosa PB, Dalsenter Y, Werle I, Platt N, Rodrigues ALS (2020) The involvement of P38/Akt/mTOR/GSK3β signaling pathways in the antidepressant-like effect of AZD6765. Pharmacol Biochem Behav 173020. https://doi.org/10.1016/j.pbb.2020.173020

Neumeyer AM, Aniit J, Chan J, Perrin JM, Murray D, Coury DL, Bennett A, Farmer J, Parker RA (2019) Identifying associations among co-occurring medical conditions in children with autism Spectrum disorders. AcadPediatr. 19(3):300–306. https://doi.org/10.1016/j.acap.2018.06.014

Niere F, Raab-Graham KF (2017) mTORC1 is a local, postsynaptic voltage sensor regulated by positive and negative feedback pathways. Front Cell Neurosci 11:152. https://doi.org/10.3389/fncel.2017.00152

Polakiewicz RD, Schiefer SM, Gingras AC, Sonenberg N, Comb MJ (1998) Mu-opioid receptor activates signaling pathways implicated in cell survival and translational control. J Biol Chem 273(36):23534–23541. https://doi.org/10.1074/jbc.273.36.23534

Puigserver P, O’Rahilly S, Muroya Y, Bouxsein-Garcia A, Lutz B, Maldonado R, Ozaita A (2009) Cannabinoid modulation of hippocampal long-term memory is mediated by mTOR signaling. Nat Neurosci 12(9):1152–1158. https://doi.org/10.1038/nn.2369

Rahi S, Gupta R, Sharma A, Mehan S (2021) Smo-Shh signaling activator purmorphamine ameliorates neurobehavioral, molecular, and morphological alterations in an intracerebroventricular propionic acid-induced experimental model of autism. Hum Exp Toxicol:9603271211013456. https://doi.org/10.1177/09063271211013456

Rai SN, Dinilashin H, Birla H, Singh SS, Zahra W, Rathore AS, Singh BK, Singh SP (2019) The role of PI3K/Akt and ERK in neurodegenerative disorders. Neurotox Res 35(3):775–795. https://doi.org/10.1007/s12640-019-0003-y

Rajdev K, Siddiqui EM, Jadaun KS, Mehan S (2020) Neuroprotective potential of solanolex in a combined model of intracerebral and intraventricular hemorrhage in rats. IBRO Rep 8:101–114. https://doi.org/10.1007/s12640-020-03001-6

Bala R, Khandha D, Mehan S, Kalra S (2015) Experimental evidence for the potential of lycopene in the management of scopolamine induced amnesia. RSC Adv. https://doi.org/10.1039/c5ra13103fEID: 2-s2.0-84940904950

Rivière JB, Mirzaz GM, O’Roak BJ, Beddaoui M, Alcantara D, Conway RL, St-Onge J, Schwartzentruber JA, Gripp KW, Nikkel SM, Worthylake T (2012a) De novo germline and postzygotic mutations in AKT3, PIK3R2 and PIK3CA cause a spectrum of related megalencephaly syndromes. Nat Genet 44(8):934–940. https://doi.org/10.1038/natgen.2012.62

Rivière JB, Mirzaz GM, O’Roak BJ, Beddaoui M, Alcantara D, Conway RL, St-Onge J, Schwartzentruber JA, Gripp KW, Nikkel SM, Worthylake T (2012b) De novo germline and postzygotic mutations in AKT3, PIK3R2 and PIK3CA cause a spectrum of related megalencephaly syndromes. Nat Genet 44(8):934–940. https://doi.org/10.1038/natgen.2012.62

Rokaya MB, Münzbergová Z, Timsina B, Bhattarai KR (2012) Rheum australe D. Don: a review of its botany, ethnobotany, and phytochemistry. J Ethnopharmacol 141(3):761–774. https://doi.org/10.1016/j.jep.2012.03.048

Ronesi JA, Huber KM (2008) Homer interactions are necessary for metabolic glutamate receptor-induced long-term depression and translational activation. J Neurosci 28(2):543–547. https://doi.org/10.1523/JNEUROSCI.5019-07.2008

Sacai H, Sakoor K, Konno K, Nagahama K, Suzuki H, Watanabe T, Kano M (2020) Autism spectrum disorder-like behavior caused by reduced excitatory synaptic transmission in pyramidal neurons of mouse prefrontal cortex. Nat Commun 11(1). https://doi.org/10.1038/s41467-020-18861-3

Saghazadeh A, Ataieinia B, Keynejad A, Abdollahizadeh A, Birbod-Mobarakeh A, Rezaei N (2019) A meta-analysis of pro-inflammatory cytokines in autism spectrum disorders: effects of age, gender, and latitude. J Psychiatr Res 115:90–102. https://doi.org/10.1016/j.jpsychires.2019.05.019

Salles MJ, Hervé D, Rivet JM, Longueville S, Millan MJ, Girault JA, Mannoury la Cour C (2013) Transient and rapid activation of Akt/GSK-3β and mTORC1 signaling by D3 dopamine receptor stimulation in dorsal striatum and nucleus accumbens. J Neurochem 125(4):532–544. https://doi.org/10.1111/jnc.12206
San Yeung K, Ip JJK, Mak CCY, Leung GKC, Tsang MHY et al (2017) Identification of mutations in the PI3K- AKT-mTOR signalling pathway in patients with macrocephaly and developmental delay and/or autism. Mol Autism 8(1):1–11

Santini E, Heiman M, Greengard P, Valjent E, Fisone G (2009) Inhibition of mTOR signaling in Parkinson’s disease prevents L-DOPA-induced dyskinesia. Sci Signal 2(80):ra36. https://doi.org/10.1126/scisignal.2006308

Sanz P, Garcia-Gimeno MA (2020) Reactive glia inflammatory signaling pathways and epilepsy. Int J Mol Sci 21(11):4096. https://doi.org/10.3390/ijms21114096

Sahu R, Kumar S, Mannel A, Alshammari A, Alharbi M, Santini E, Heiman M, Greengard P, Valjent E, Fisone G (2009) San Yeung K, Tso WWY, Ip JJK, Mak CCY, Leung GKC, Tsang MK (2015) Characterizing autism spectrum disorders by key biological marker of sporadic AD. Neurology 54(6):1297–1304

Shams S, Foley KA, Kavaliers M, MacFabe DF, Ossenkopp KP (2019) Systemic treatment with the enteric bacterial metabolic product propionic acid results in reduction of social behavior in juvenile rats: contribution to a rodent model of autism spectrum disorder. Dev Psychobiol 61(5):688–699

Sharma A, Mehan S (2021) Targeting PI3K-AKT/mTOR signaling in the prevention of autism. Neurochem Int 147:105067. https://doi.org/10.1016/j.neuint.2021.105067

Sharma N, Upadhyay S, Shandilya A, Sahu R, Singh A, Rajkhowa B, Mehan S (2021) Neuroprotection by solanesol against ethidium bromide-induced multiple sclerosis-like neurobehavioral, molecular, and neurochemical alterations in experimental rats. Phytochem Plus 1(4):100051. https://doi.org/10.1016/j.phyplu.2021.100051

Sharma R, Rahi S, Mehan S (2019) Neuroprotective potential of solanesol in intracerebroventricular propionic acid induced experimental model of autism: insights from behavioral and biochemical evidence. Toxicol Rep 6:1164–1175. https://doi.org/10.1016/j.toxrep.2019.10.019

Shultz SR, MacFabe DF, Ossenkopp KP, Scratch S, Whelan J, Taylor R, Cain DP (2008) Intracerebroventricular injection of propionic acid, an enteric bacterial metabolic end-product, impairs social behavior in the rat: implications for an animal model of autism. Neuropharmacology. 54(6):901–911. https://doi.org/10.1016/j.neuropharm.2008.01.013

Singh A, Upadhyay S, Mehan S (2021) Inhibition of c-JNK/p38MAPK signaling pathway by Apigenin prevents neurobehavioral and neurochemical defects in ethidium bromide-induced experimental model of multiple sclerosis in rats: Evidence from CSF, blood and brain samples. Phytochem Plus 1(4):100139

Singh D, Rawat MS, Semalty A, Semalty M (2013) Chrysophanol–phospholipid complex. J Thermal Anal Calorim 111(3):2069–2077

Soltani A, Lebrun S, Carpenter G, Zunino G, Chantepie S, Maïza A, Bozzi Y, Desnos C, Darchen F, Stettler O (2017) Increased signaling by the autism-related Engrailed-2 protein enhances dendritic branching and spine density, alters synaptic structural rearrangement, and exaggerates protein synthesis. PLoS One 12(8):e0181350. https://doi.org/10.1371/journal.pone.0181350

Su S, Wu J, Gao Y, Luo Y, Yang D, Wang P (2020) The pharmacological properties of chrysophanol, the recent advances. Biomed Pharmacother 125:110002

Subramanian M, Timmerman CK, Schwartz JL, Pham DL, Meffert MK (2015) Characterizing autism spectrum disorders by key biochemical pathways. Front Neurosci (9):313. https://doi.org/10.3389/fnins.2015.00313

Takei N, Nawa H (2014) mTOR signaling and its roles in normal and abnormal brain development. Front Mol Neurosci 7:28. https://doi.org/10.3389/fnmol.2014.00028

Thomas RH, Foley KA, Mepham JR, Tichenoff LJ, Plossmayer F, MacFarbe DF (2010 Apr) Altered brain phospholipid and acyl-carnitine profiles in propionic acid infused rodents: further development of a potential model of autism spectrum disorders. J Neurochem 113(2):515–529. https://doi.org/10.1111/j.1471-4159.2010.06614.x

Thomas RH, Meeking MM, Mepham JR, Tichenoff L, Plossmayer F, Liu S, MacFarbe DF (2012) The enteric bacterial metabolite propionic acid alters brain and plasma phospholipid molecular species: further development of a rodent model of autism spectrum disorders. J Neuroinflammation 9:153. https://doi.org/10.1186/1742-2094-9-153

Tiwari A, Khera R, Rahi S, Mehan S, Makeen HA, Khormi YH, Rehman MU, Khan A (2021) Neuroprotective effect of α-Mangostin in the ameliorating propionic acid-induced experimental model of autism in Wistar rats. Brain Sci 11(3):288. https://doi.org/10.3390/brainsci11030288

Tong X, Zhang J, Shen M, Zhang J (2020) Silencing of tenascin-C inhibited inflammation and apoptosis via PI3K/Akt/NF-κB signaling pathway in subarachnoid hemorrhage cell model. J Stroke Cerebrovasc Dis 29(1):104485. https://doi.org/10.1016/j.jstrokecerebrovasdis.2019.104485

Wang J, Lv P (2021) Chrysophanol inhibits the osteoglycin/mTOR and activats GABABR signaling to reduce viability and proliferation of malignant meningioma cells. Bioengineered 12(1):755–762

Wang WP, Zhang MY (2017) Role for target of rapamycin (mTOR) signal pathway in regulating neuronal injury after intracerebral hemorrhage. Cell Physiol Biochem 41(1):145–153. https://doi.org/10.1159/000455983

Wang Z, Fang J, Xiao J (2019) Correlation of the expression of inflammatory factors with expression of apoptosis-related genes Bax and Bcl-2, in burned rats. Exp Ther Med 17(3):1790–1796

Workman ER, Niere F, Raab-Graham KF (2013) mTORC1-dependent protein synthesis underlying rapid antidepressant effect requires GABABR signaling. Neuropharmacology. 73:192–203. https://doi.org/10.1016/j.neuropharm.2013.05.037

Yu N, Hu S, Hao Z (2018) Beneficial effect of stachydrine on the traumatic brain injury induced neurodegeneration by attenuating inflammatory factors with expression of apoptosis-related genes Bax and Bcl-2, in burned rats. Exp Ther Med 17(3):1790–1796

Zhang J, Kang H, Wang L, Zhao X (2018) Chrysophanol ameliorates the expressions of Akt/mTOR/PI3K and TLR4/NFκ-B pathway in regulating neuronal injury after intracerebral hemorrhage induced neurodegeneration by attenuating inflammation and apoptosis-related genes. J Transl Neurosci 9(1):175–182

Zhu X-Z, Kim GH, Tan J-W, Riso AE, Sun Y, Xu EY, Xu B (2020) Elevated protein synthesis in microglia causes autism-like synaptic and behavioral aberrations. Nat Commun 11(1). https://doi.org/10.1038/s41467-020-15530-3

Yadav RK, Mehan S, Sahu R, Kumar S, Khan A, Makeen HA, Al Bratty M (2022) Protective effects of apigenin on methylmercury-induced behavioral/ neurochemical abnormalities and neurotoxicity in rats. Hum Exp Toxicol 41:09603271221084276

Yu N, Hu S, Hao Z (2018) Beneficial effect of stachydrine on the traumatic brain injury induced neurodegeneration by attenuating the expressions of Akt/mTOR/PI3K and TLR4/NFκ-B pathway. Transl Neurosci 9(1):175–182

Zhang J, Kang H, Wang L, Zhao X (2018) Chrysophanol ameliorates high-fat diet-induced obesity and inflammation in neonatal rats. Die Pharmazie 73(4):228–233

Zhang J, Yan C, Wang S, Hou Y, Xue G, Zhang L (2014a) Chrysophanol ameliorates lead exposure-induced injury to hippocampal
neurons in neonatal mice. Neural Regen Res 9(9):924–930. https://doi.org/10.4103/1673-5374.133141
Zhang K, Liu J, You X, Kong P, Song Y, Cao L, Yang S, Wang W, Fu Q, Ma Z (2016) P2X7 as a new target for chrysophanol to treat lipopolysaccharide-induced depression in mice. Neurosci Lett 613:60–65. https://doi.org/10.1016/j.neulet.2015.12.043
Zhang M, Jiao J, Hu X, Yang P, Huang Y, Situ M, Guo K, Cai J, Huang Y (2020) Exploring the spatial working memory and visual perception in children with autism spectrum disorder and general population with high autism-like traits. PLoS One 15(7):e0235552. https://doi.org/10.1371/journal.pone.0235552
Zhang N, Zhang X, Liu X, Wang H, Xue J, Yu J, Kang N, Wang X (2014b) Chrysophanol inhibits NALP3 inflammasome activation and ameliorates cerebral ischemia/reperfusion in mice. Mediat Inflamm 2014:370530. https://doi.org/10.1155/2014/370530
Zhao Y, Fang Y, Li J, Duan Y, Zhao H, Gao L, Luo Y (2016a) Neuroprotective effects of Chrysophanol against inflammation in middle cerebral artery occlusion mice. Neurosci Lett (630):16–22. https://doi.org/10.1016/j.neulet.2016.07.036
Zhao Y, Fang Y, Zhao H, Li J, Duan Y, Shi W, Huang Y, Gao L, Luo Y (2018a) Chrysophanol inhibits endoplasmic reticulum stress in cerebral ischemia and reperfusion mice. Eur J Pharmacol (818):1–9. https://doi.org/10.1016/j.ejphar.2017.10.016
Zhao Y, Huang Y, Fang Y, Zhao H, Shi W, Li J, Duan Y, Sun Y, Gao L, Luo Y (2018b) Chrysophanol attenuates nitrosative/oxidative stress injury in a mouse model of focal cerebral ischemia/reperfusion. J Pharmacol Sci 138(1):16–22
Zhao Y, Fang Y, Li J, Duan Y, Zhao H, Gao L, Luo Y (2016b) Neuroprotective effects of Chrysophanol against inflammation in middle cerebral artery occlusion mice. Neurosci Lett (630):16–22. https://doi.org/10.1016/j.neulet.2016.07.036
Zheng Z, Zhang L, Zhu T, Huang J, Qu Y, Mu D (2016 Aug) Peripheral brain-derived neurotrophic factor in autism spectrum disorder: a systematic review and meta-analysis. Sci Rep 10(6):31241. https://doi.org/10.1038/srep31241

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.