RESEARCH PAPER

Ferulic Acid Ameliorates Sodium Valproate Induced Autism-Like Behaviors in Male Rat Pups

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ABSTRACT

The present investigation was conducted for exploring neuroprotective activity of ferulic acid in autistic male rat pups. Wistar female rats were injected with sodium valproate (400 mg/kg, *s.c.*) on gestational day 13 for inducing autism-like features in their pups. Ferulic acid (15, 30 -and 60 mg/kg) was orally administered to autistic male pups for 35 consecutive days (from postnatal day 24 to 58). Various behavioural paradigms like tail immersion test, actophotometer, tail suspension test, elevated zero maze, social interaction test and Morris's water maze were employed. On postnatal day 58, animals were euthanized for obtaining brain and blood samples for biochemical estimations. Cerebellum part of their brain was used for histopathological studies. Reduced body weight at birth, prolonged eye-opening duration and impairment of motor coordination in male pups indicated induction of autism in them. There was significant reduction in pain sensitivity and increase of locomotion; depressive and anxious behaviors, impaired learning and memory; and deficits of social interaction in autistic pups. There was significant increase of oxidative stress (elevated malondialdehyde and nitrite levels; and decreased brain GSH and catalase activity), acetylcholinesterase and MAO-A activities in brain of pups. Plasma corticosterone levels were also significantly elevated in autistic pups. Ferulic acid significantly reversed above mentioned behavioural, biochemical and histopathological alterations in autistic male rat pups. Thus, ferulic acid significantly ameliorated autism- like behaviours in male rat pups exposed prenatally to sodium valproate...

Keywords: Autism spectrum disorder, sodium valproate, ferulic acid

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INTRODUCTION

Autism spectrum disorder (ASD) is a development disorder of nervous system marked by constant problems in social communication, confined interests, and repetitive behaviors[1]. The prevalence of ASD has increased markedly, with recent data indicating a diagnosis rate of approximately 1 in 31 children in the United States as of 2025, which equates to about 3.2% of 8-year-old children, and somewhat lower but rising estimates globally. Males are diagnosed more frequently than females, with ASD reported across diverse racial and ethnic groups[2]. The aetiology of autism is multifactorial, involving intricate genetic and environmental interactions. Genetic contributions include mutations and chromosomal aberrations affecting neurodevelopment, while environment risk factors include parental age, prenatal exposure to certain medications, and maternal metabolic disturbances[3]. Comorbidity is highly prevalent in ASD, with individuals frequently exhibiting coexisting conditions such as attention-deficit hyperactivity disorder, anxiety, epilepsy, intellectual disabilities, and gastrointestinal disturbances. These comorbidities complicate diagnosis and influence the severity of ASD symptoms[4]. The pathophysiology of ASD includes aberrant synaptic connectivity, neuroinflammatory processes, and dysregulation of key neurotransmitter systems, notably involving gamma-aminobutyric acid, glutamate, dopamine, and serotonin. Brain regions such as the prefrontal cortex and cerebellum are often implicated, with dysfunction in these areas contributing to atypical neurodevelopmental trajectory seen in autism[5].

Aripiprazole and risperidone have been approved by US FDA for management of some signs and symptoms associated with autism. These drugs suppress only self-mutilation, aggressiveness, irritability and stereotype behaviors[6]. Aripiprazole and risperidone have side effects such as increased appetite, weight gain, drowsiness, fatigue and hyperprolactinemia[7, 8]. Herbal drugs now a days are used as a safer alternative in managing different psychiatric illness and neurodegenerative diseases not responding to currently available medications[9]. Plant based compounds like sulforaphane, cannabidiol and luteolin have been reported in clinical studies to exert significant beneficial effect in controlling the core symptoms of autism spectrum disorder[10-14]

Ferulic acid is plant-derived phenolic compound that has demonstrated substantial neuroprotective effects in various experimental rodents' models. Pharmacological activities reported with ferulic acid include antioxidant[15], anti-inflammatory[16], anti-Alzheimer[17, 18], anti-Parkinsonian[19],

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anti-Huntington[20], memory enhancing[21], anti-epileptic[22], anxiolytic[23, 24] and antidepressant[25]. In addition to these activities, ferulic acid has also shown anti-autistic like activity in zebra fish through its antioxidant, anti-inflammatory, and anti-apoptotic properties via modulation of phosphoinositide 3-kinase/AKT/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway[26]. Hydroalcoholic extract of *Ferula gummosa* containing ferulic acid (along with other phytochemicals) as one of active constituents has been reported to exert beneficial effect against autism in maternally separated mice[27]. But, effects of ferulic acid against sodium valproate induced autism-like behaviours in rodents have not been explored till date. Thus, the present investigation has been conducted for evaluation of neuroprotective potential of ferulic acid in male rat pups exposed prenatally to sodium valproate.

2 MATERIALS AND METHODS

2.1 *Drugs and chemicals*

Ferulic acid, donepezil hydrochloride, fluoxetine hydrochloride, 5-hydroxy-tryptamine hydrochloride, aripiprazole and 5,5'-Dithiobis (2-nitrobenzoic acid), employed in this study, were obtained from Sigma-Aldrich (USA). Injections of diazepam (Calmpose®, Sun Pharmaceutical Industries Ltd., India) and sodium valproate (Valprol®, Intas Pharmaceutical Ltd., India) were used. All other chemicals of analytical grade were used in this study. The solutions of drugs and chemicals were prepared freshly before use.

2.2 *Preparation of drug solution*

Ferulic acid was suspended in normal saline containing 0.5% w/v carboxy methylcellulose. Donepezil and fluoxetine were dissolved in 0.9% w/v solution of sodium chloride. Injections of diazepam and fluoxetine were diluted in normal saline. Aripiprazole was suspended in 2.5% v/v tween 80.

2.3 *Experimental animals*

Male and female Wistar albino rats (3-4 months old; weighing 160-220 g) were procured from DFSAH, LUVAS, Hisar, Haryana. These were mated and pregnant rats were used for autism induction in their pups. Only male pups were selected for the experiments, since males are more affected with autism than females[28-30]. The research protocol and number of rats to be used for carrying out this study were approved by Institutional Animals' Ethics Committee (IAEC) in its meeting held on 10th February, 2022 (Minutes Letter No. IAEC/2022/10-18). The animals were housed in groups (6 animals/cage) in an air-conditioned room having temperature 23 ± 2 °C. The animals were acclimatized to the laboratory conditions for 7 consecutive days. These animals were allowed unrestricted access to food and water, except that food was withdrawn for two hours prior to and following drug administration. All experimental protocols were conducted in compliance with the guidelines issued by CCSEA, New Delhi.

2.4 *Dose selection*

The dose of sodium valproate[31], ferulic acid[32], aripiprazole[33], fluoxetine[34], donepezil[35] and diazepam[36] was selected based on earlier studies. Volume of vehicle/drugs administered to pregnant rats was 0.5 ml/100 g and it was 1 ml/100 g of body weight for rat pups.

2.5 *Induction of autism*

Female rats were screened for regular estrous cycle through vaginal smear analysis following a seven-day acclimatization period. Animals observed in the estrous phase were mated (two females and one male were housed together overnight). Successful mating was confirmed in the next morning by presence of sperms in vaginal smear examination and this day was considered as day 1 of gestation period[37]. Sodium valproate (400 mg/kg. s.c.) was injected on 13th day of gestation period to induce autism, whereas control animals received normal saline. Pregnant females were then housed individually in separate polypropylene cages (43 x 27 x 15 cm^3) on 18th day for parturition and rearing of litters. Body weight of pups was noted at birth, negative geotaxis was observed on postnatal day 8 and 12, and eye-opening time was recorded on postnatal day 13 to 17[38, 39]. Offspring were weaned on postnatal day 23, after which males and females were separated.

2.6 *Experimental design*

Following 9 groups of male rat pups were constituted (n = 6 each group).

Group I (normal control pups): These were maintained on normal diet.

Autistic rat pups

Group II (Autistic control pups): Autistic rat pups received vehicle (normal saline having carboxy methyl cellulose, 0.5% w/v) through oral route during postnatal day (PND) 24 to 58.

Group III, IV and V: Autistic rat pups received ferulic acid (15, 30 and 60 mg/kg; *p.o.*) during PND 24 to 58.

Group VI: Autistic rat pups received standard anti-psychotic drug, aripiprazole through intraperitoneal route (1 mg/kg/day) during PND 24 to 58.

Group VII: Autistic rat pups received standard anti-depressant drug, fluoxetine (10 mg/kg; *i.p.*) during PND 24 to 58.

Group VIII: Autistic rat pups received standard anti-anxiety drug, diazepam (2 mg/kg; *i.p.*) during PND 24 to 58.

Group IX: Autistic rat pups received donepezil (0.75 mg/kg; *i.p.*), the standard memory enhancing drug, during PND 24 to 58.

Behavioral evaluations were conducted between PND 47 and 57. During these assessments, vehicle or drug treatments were administered one hour prior to initiation of each behavioral paradigm. The sequence of testing included: tail immersion test for nociceptive sensitivity on PND 47, assessment of locomotor activity using an actophotometer on PND 48, tail suspension test for depressive-like behavior on PND 49, elevated zero maze for anxiety related behavior on PND 50, and Morris water maze for spatial learning and memory between PND 52 and 56. Social interaction testing was performed on PND 57. On PND 58, rat pups were euthanized by cervical dislocation under light anaesthesia using chloroform so as to minimise pain to the animals (Figure 1).

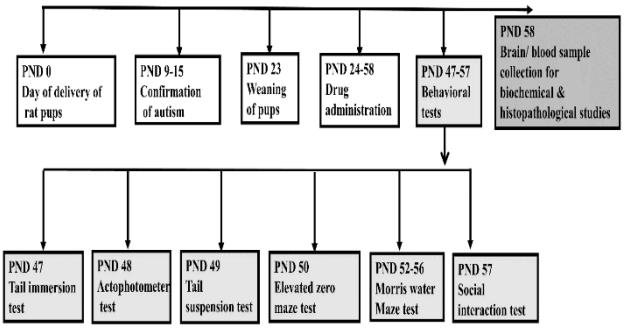

Figure 1. Schematic representation of experimental protocol

2.7 *Behavioral tests*

2.7.1 Confirmatory tests of induction of autism in rat pups

The new born rat pups were carefully weighed after delivery. Similarly, eyelid opening time of these pups was observed on postnatal day 13 to 17[38]. Negative geotaxis is a vestibular reflex elicited in response to gravitational stimuli and serves as an indicator of sensorimotor development in neonatal rats. For this assessment, a glass platform (25 x 25 cm^2) covered with a rough paper was employed. Both autistic control and normal control pups were subjected to this test on postnatal days 8 and 12. Each pup was positioned on a 45° inclined plane, and the tendency to rotate 180° was recorded[40].

2.7.2 Tail immersion test

The tail immersion test is a widely utilized model for evaluating effect of compounds on acute nociceptive pain induced by thermal stimuli. Each rat pup was gently restrained in a semi-cylindrical holder. The distal portion of the tail, which extended freely beyond the restraint device, was submerged in warm water bath maintained at 55 ± 0.5°C. The latency to tail withdrawal was recorded as the nociceptive response. To prevent potential tissue damage, a maximum cut-off time of 10 seconds was imposed. Tail-flick latencies of pups were noted 1 h after oral administration of test drug/vehicle[41].

2.7.3 Locomotor activity

Horizontal locomotor activity of each rat pup was recorded using an actophotometer (INCO Pvt. Ltd., Ambala, Haryana). The apparatus is equipped with photoelectric sensors connected to a digital counter, which records activity each time the animal's movement interrupts the light beam. Locomotor activity was quantified over a 5-minute testing session[42].

2.7.4 Tail suspension test

TST (Tail suspension test) is one of the most common paradigms, used to explore effects of substances on depressive behavior in rodents. Each rat pup was suspended individually from table-edge, 50 cm above the ground level. The animal was suspended by using adhesive tape on the distal part of tail (approximately 1 cm from tip of the tail). A period of 6 min. was employed to record immobility of each animal. The animal showing no body movement and hanging passively was considered immobile. The experiment was performed in dim lighted room. Each rat pup was subjected to the test once only[43, 44].

2.7.5 Elevated zero maze

Elevated zero maze is commonly used to assess anxiety-like behaviour of rats and mice. This maze is having white/black annular ring having an outer diameter of 45 cm and inner diameter of 30 cm; with an elevation of 40 cm above the floor. The runway ring (6 cm wide) for exploration by the rat pup consists of 4 opposite quadrants, two open quadrants with no walls and two enclosed quadrants with walls of 12 cm height. The open quadrants have a ridge of 2-3 mm to prevent the rat pup to fall off. Each rat pup was placed in the closed arm with face towards open arm. It was permitted 5 minutes for exploring the maze. Total time spent and numbers of entries by the animal in open arms of the maze were measured. Ethanol (70% v/v) was used to clean the maze between trials to prevent the olfactory cues for the next animal being tested[45].

2.7.6 Morris water maze

This maze was constructed of aluminium sheet and circular in shape with 60 cm diameter and 25 cm height. It was filled with opaque water (made by using non-toxic white dye) up to 20 cm height and water temperature was adjusted to 25 ± 2° C. There were two black threads fixed on the edge of the maze to divide the maze into 4 equivalent quadrants, named as Q1, Q2, Q3 and Q4. A white coloured metallic platform (6 cm × 6 cm) was kept 1 cm beneath the water surface inside Q4 quadrant. The same position was selected for placing this platform during training sessions. Each rat pup was placed in one of the quadrants facing the wall of pool during training. Each rat pup undergone 4 successive training sessions per day with an interval of five minutes for four successive days (day 52 to 55 of the study). The initial position of each rat pup during training sessions for 4 successive days was as follows:

First day: Quadrant 1; 2; 3 and 4
Second day: Quadrant 2; 3; 4 and 1
Third day: Quadrant 3; 4; 1 and 2
Fourth day: Quadrant 4; 1; 2 and 3

Each rat pup was allowed maximum time of 120 sec to find the platform hidden under the water. On reaching the platform, a time of 20 sec was allowed for each rat pup to stay there, before being gently dried with a towel and returned to its home cage. The rat pup was guided to the platform using a glass rod if it could not search the same within 120 seconds. The rat pup was subsequently permitted to stay on the platform for 20 seconds. The time used by each rat pup to find hidden platform was noted as the escape latency (EL).

On reaching the platform, the animal was allowed to remain on the platform for 20 seconds before being gently dried with a towel and return to its home cage. The rat pup was guided to the platform using a glass rod if it was unable to locate the hidden platform within 120 seconds, and subsequently allowed to stay on it for 20 seconds. The duration taken by each rat pup to locate the hidden platform was recorded as the escape latency (EL).

The platform was removed on 5th day (56th day of the study) from the pool, for evaluating retention of learned behaviour.

Each rat pup was placed one by one in any of the 3 quadrants (Q1, Q2 and Q3) for exploration of Q4 within 120 seconds. The time spent by the rat pup in Q4 (target quadrant) was recorded. The spatial orientation of the water maze in relation to surrounding objects was maintained constant throughout the duration of the study. The observer's position was same during all the trials[46, 47].

2.7.7 Social interaction test

The social interaction test was conducted in a plastic cage having dimensions of 43 x 27 x 15 cm^3. To enhance responsiveness during training, rat pups were housed separately the night prior to the experiment. Following drug or vehicle administration, pairs of pups were placed together in the test cage for a session of 10-min. Social interaction was examined by monitoring specific behaviors, including pinning (one pup lying on its back while the other places forepaws on top), following, mutual grooming, physical contact, anogenital sniffing and general body sniffing[36, 48].

The social interaction score in percentage was calculated using the formula given below:

$$\text{Social interaction score (\%)} = \frac{\text{Number of seconds spent in interaction X 100}}{\text{600 seconds (observation time)}}$$

2.8 Biochemical estimations

The whole brain was isolated after euthanizing the rat pups by cervical dislocation. The samples of brain were carefully rinsed with an ice-cold buffered solution (pH 7.4) prepared by using sucrose (0.25 Moles), Tris (0.1 Moles), and EDTA (0.02 Moles) prior to weighing. Each brain was then divided into two equal portions:

Part A: One portion of the brain tissue was homogenized in nine volumes of ice-cold sucrose-Tris- EDTA buffer (pH 7.4) using a glass homogenizer placed on ice bath. The homogenate was centrifuged at 2500 r.p.m. for 10 minutes at 4°C in a refrigerated centrifuge (Remi Elektrotechnik Ltd., Mumbai). The precipitates were disposed. The supernatant fluid used for re-centrifuged at speed of 12000 r.p.m. at 4°C for another 20 min. The resultant mitochondrial portion in the form of pellets were taken for determination of MAO-A (Monoamine oxidase A) enzyme activity, while the corresponding supernatant was employed for the determination of MDA (lipid peroxidation), catalase, nitrite, GSH (reduced glutathione) and protein content.

Part B: The second portion of brain tissue was homogenized in nine volumes of 0.1 M phosphate buffer (pH 8) using a glass homogenizer on an ice bath. The homogenate was subjected to centrifugation at speed of 3,000 r.p.m at 4°C for 10 min., and the supernatant fluid collected was employed for determining acetylcholinesterase activity[49]

2.8.1 Measurement of MAO-A activity

The mitochondria fraction obtained as described in Part A was washed two times using sucrose-Tris-EDTA buffer and subsequently re-mixed in nine parts of ice-cold sodium phosphate buffer (having pH 7.4) containing 0.32 Moles sucrose. The suspension was gently mixed for 20 minutes at 4 °C followed by centrifugation at 15,000 r.p.m. using a cooling centrifuge for a duration of 30 minutes at 0 °C. The resulting pellet was re-mixed in cold solution of sodium-phosphate buffer. For enzymatic assay, 150 µl of this suspension was added to a quartz cuvette containing 2.75 ml of sodium phosphate buffer along with 100 µl of 5-HT (serotonin, 4mM) for initiation of reaction. The changes of absorbance were measured for 5 minutes at 280 nm using a blank solution (having 5-HT and phosphate buffer) employing a UV-Visible spectrophotometer (GENESYS 180, India) [50].

2.8.2 Measurement of protein concentration

The concentration of total protein in brain homogenates was measured using Biuret method with commercially available reagent kit (Erba Mannheim, Germany). Absorbance at 546 nm was recorded employing a semi-automatic autoanalyzer (Chrm 5plus- V2, Erba Mannheim, Germany).

2.8.3 Measurement of malondialdehyde

MDA (Malondialdehyde) levels indicate lipid peroxidation. For the assay, 0.5 ml of the supernatant (obtained as described in Part A) was incubated with 0.5 ml Tris-HCl buffer for 2 hours at 37 °C. Following incubation, 1 ml of TCA (tri-chloro acetic acid, 10% w/v), added and resulting mixture subjected to centrifugation at speed of 1,000 r.p.m for a period of 10 minutes. From resulting supernatant, 1 ml added to test tube having 1 ml of TBA (thiobarbituric acid, 0.67% w/v) followed by keeping it over a water bath (containing boiling water) for 10 min. Upon cooling, 1 ml of double distilled water was mixed. Absorbance of reaction mixture was recorded at 532 nm using a UV-visible spectrophotometer (GENESYS 180, India). MDA levels were reported as nano moles per milligram of protein[51].

2.8.4 Measurement of reduced glutathione

Reduced glutathione (GSH) levels in brain tissue were determined as follows: 1 ml of the supernatant (obtained from Part A) was added to 1 ml of of 4 % sulfosalicylic acid in a test tube following incubation for one hour at 4°C. This mixture was further subjected to centrifugation at speed of 1,200 r.p.m. at 4°C for 15 minutes. From resulting supernatant fluid, 100 µl was added to 2.7 ml of phosphate buffer (having pH 8) which was followed by adding 200 µl DTNB [10 mM, 5,5'-Dithiobis (2-nitrobenzoic acid)]. Absorbance of resultant yellow coloured compound was noted at 412 nm using a UV-visible spectrophotometer (GENESYS 180, India). GSH concentration was expressed as nmol/mg protein[52].

2.8.5 Measurement of catalase activity

For assessment of activity of brain catalase, hydrogen peroxide solution [0.3 ml of H_2O_2 in 50 ml of phosphate buffer (0.05M, pH 7)] used as substrate. For the assay, 20 µl of the supernatant (obtained as described in Part A) was mixed with 0.98 ml substrate. Absorbance at 230 nm was noted down employing a UV-visible spectrophotometer (GENESYS 180, India) after 1 minute and again after 6 minutes of incubation. Catalase activity was expressed as µmol of H_2O_2 decomposed per minute per mg of protein[53].

2.8.6 Measurement of nitrite level

The concentration of nitrite in brain was determined by using Griess reagent, which was prepared by mixing same volumes of sulphanilamide (1 % w/v) in phosphoric acid (5% v/v) and N-naphthyl ethylenediamine dihydrochloride

(0.1 % w/v) in distilled water. For performing assay, 1 ml of Greiss reagent was added to 1 ml of the supernatant (obtained from Part A of brain homogenate) followed by incubation for 10 minutes at room temperature to allow formation of coloured complex, absorbance of which was measured at 543 nm employing a UV-visible spectrophotometer (GENESYS 180, India). Nitrite concentration was calculated and expressed as µg/mg of protein[54].

2.8.7 Measurement of acetylcholinesterase

Acetylcholinesterase (AChE) activity was determined by using Part B of brain. For the assay, 0.4 ml of the supernatant was mixed with 2.6 ml phosphate buffer followed by addition of 100 µl of 5, 5'-dithiobis-(2-nitrobenzoic acid) reagent. Absorbance of this was noted at 412 nm employing a UV-visible spectrophotometer (GENESYS 180, India). Once a stable baseline absorbance was achieved, it was set to zero, followed by addition of acetylthiocholine iodide solution (20 µl) for initiation of the reaction. Absorbance was measured again for a total period of 5 minutes, and the change in absorbance per minute was used to calculate the rate of substrate hydrolysis by AChE according to the standard formula.

$$R = \frac{5.74 \times 10^{-4} \times \Delta ABS}{C_0}$$

R indicates substrate hydrolysis rate in µmol/minute/gram of wet tissue; ΔABS indicates absorbance change per minute; C_0 indicates tissue sample concentration in milligram/millilitre[55].

2.8.9 Measurement of plasma corticosterone

For isolation of brain samples, blood samples were also collected by cardiac puncture. Plasma was separated immediately by using a refrigerated centrifuge. Corticosterone levels were determined in plasma samples by using colorimetric method. Briefly, 1 ml of plasma sample and 1 ml of ethanol were added to test tubes containing 0.5 ml of 0.1 % (w/v) N,N-dimethyl-4-nitrosoaniline prepared in ethanol. The tubes were then placed in an ice-cold water bath for 5 minutes, followed by addition of 0.1 N sodium hydroxide (0.5 ml). After sealing the test tubes with cotton plugs, these were maintained at zero° C for 5 hours and protected from light. This was followed by addition of 2 ml of Clark and Lubs buffer of pH 9.8, 5 ml ethanol containing 0.1% phenol, and 0.5 ml of 1 % w/v solution of potassium ferricyanide. The resulting mixture then, incubated for 10 minutes at room temperature, followed by measurement of absorbance at 650 nm by employing a UV-visible spectrophotometer (GENESYS 180, India)[56, 57].

2.9 Histopathological evaluation

The cerebellum is recognized as a critical brain region involved in the neuropathology of autism spectrum disorder, with structural abnormalities in this region associated with an increased likelihood of autistic phenotypes[58]. For histopathological assessment, the cerebellar region from the brain of one animal per group was dissected and preserved in 10 % neutral buffered formalin. Slides of cerebellar parts were prepared at Nalwa pathology laboratory, Hisar (Haryana). The prepared slides were subsequently examined by a veterinary pathologist to evaluate histopathological alterations in cerebellum.

2.10 Statistical analysis

The data of various behavioural and biochemical studies were denoted as Mean ± standard error mean. Statistical analyses were performed using one-way analysis of variance (ANOVA), following Tukey-Kramer multiple comparison test. Unpaired t-test was used for comparison of data for normal and autistic pups for confirmation of induction of autism. Data were processed with GraphPad InStat software (version 3.10). A value of $p<0.05$ was taken as statistically significant.

3 RESULTS

3.1 Preliminary tests for confirmation of induction of autism

In autistic pups, there was significant reduction in body weight ($p<0.0001$) at birth, increase in eye opening time ($p<0.0001$), and impaired motor coordination as indicated by increased negative geotaxis score ($p<0.05$ on day 8 and $p<0.01$ on day 12), when compared to normal control pups (Figures 2 A to 2 D).

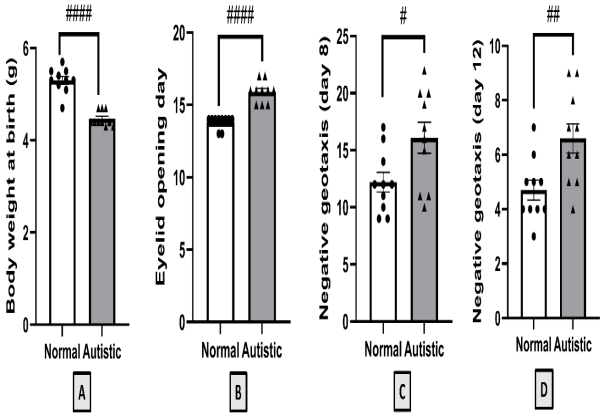

Figure 2. Preliminary tests for confirmation of induction of autism in rat pups (A) Body weight of rat pups at the time of birth; t = 7.797, $p<0.0001$; (B) Eyelid opening time of rat pups, t = 7.814, $p<0.0001$; (C and D) Negative geotaxis score of rat pups on postnatal day 8 (t = 2.426, p = 0.026) and 12 (t = 2.9, p = 0.0094). FA15 = ferulic acid (15 mg/kg); FA30 = ferulic acid (30 mg/kg); FA60 = ferulic acid (60 mg/kg). Values mentioned in the figures are Mean ± SEM. n = 10 in each group. Analysis of data was performed by using unpaired t-test.

# indicates $p<0.05$; ## indicate $p<0.01$; #### indicates $p<0.0001$ vs normal control pups

3.2 Effect of ferulic acid on tail withdrawal latency in rat pups

Autistic control pups showed a significant increase in tail withdrawal latency ($p<0.001$) compared to normal control, indicating reduction of pain sensitivity. Treatment with ferulic acid (60 mg/kg) significantly reduced withdrawal latency in autistic pups ($p<0.001$) relative to autistic control pups, indicating reversal of hypoalgesia. However, the lowest dose (15 mg/kg) and medium-dose (30 mg/kg) of

ferulic acid did not show any significant effect on pain sensitivity (Figure 3-A).

3.3 Effect of ferulic acid on locomotor activity of rat pups using actophotometer

Autistic control pups demonstrated a significant rise in locomotor activity compared to normal controls ($p<0.001$). Ferulic acid (15, 30 and 60 mg/kg; *p.o.*) administration exhibited significant decrease in locomotor activity of autistic pups ($p<0.001$) compared to autistic control pups. This observed effect of ferulic acid was comparable to that of aripiprazole, used as a standard drug in this study (Figure 3-B).

3.4 Effect of ferulic acid on immobility period rat pups in tail suspension test

Immobility duration was significantly increased in autistic control pups in comparison to normal control pups ($p<0.001$), reflecting the induction of depressive-like behavior. Administration of ferulic acid (30 and 60 mg/kg) as well as fluoxetine (10 mg/kg), significantly reduced immobility time of autistic pups relative to autistic control pups ($p<0.01$; $p<0.01$ and $p<0.001$ respectively), indicating attenuation of depressive-like behavior (Figure 3-C).

3.5 Effect of ferulic acid and diazepam on anxiety-like behaviour in autistic rat pups using elevated zero maze

Autistic control pups demonstrated a significant reduction in both time spent and total number of entries into the open arms on comparing to normal control pups ($p<0.001$), indicative of rise of anxiety-like behaviour. Treatment with ferulic acid (15, 30 and 60 mg/kg) as well as diazepam significantly improved time spent and total number of entries in open arms in autistic pups compared to autistic control pups ($p<0.001$), reflecting anti-anxiety effects of these drugs (Figures 3-D and E).

3.6 Effect of ferulic acid on social behaviour of autistic rat pups

Prenatal sodium valproate exposure resulted in a significant reduction in social interaction scores (%) in autistic control pups compared to normal control ($p<0.001$), confirming social behaviour deficits. Treatment with ferulic acid (30 and 60 mg/kg) and standard drug, aripiprazole significantly ($p<0.001$) improved social interaction scores in autistic pups relative to autistic control. However, treatment with the lowest dose of ferulic acid (15 mg/kg) produced lesser significant effect ($p<0.01$) on social interaction score (Figure 3-F).

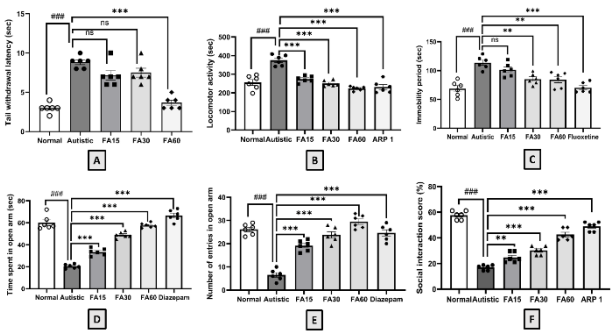

Figure 3. Effects of ferulic acid on (A) tail withdrawal latency of rat pups using tail immersion test: $F(4,25) = 34.31$; (B) locomotor activity of rat pups using actophotometer: $F(5, 30) = 27.85$; (C) immobility period of rat pups in tail suspension test: $F(5,30) = 14.64$; (D) time spent in open arms of elevated zero maze by rat pups: $F(5,30) = 119.67$; (E) number of entries in open arms of elevated zero maze by rat pups: $F(5,30) = 50.83$; (F) social behaviour: $F(5,30) = 117.50$. FA15/30/60 = ferulic acid 15/30/60 mg/kg; ARP 1 = Aripiprazole 1 mg/kg. Here, values are written as Mean ± SEM. n = 6 in each group. Analysis of data was performed using one-way analysis of variance followed by Tukey-Kramer test for multiple comparison.

indicates $p<0.001$ vs. normal control pups

** indicates $p<0.01$, *** indicates $p<0.001$ vs. autistic control pups

3.7 Effect of ferulic acid on escape latency (EL) and time spent in target quadrant (TSTQ) by rat pups using Morris's water maze

Autistic control pups exhibited significant ($p<0.001$) deficits in learning and memory, evidenced by prolonged EL across all four training days and reduced TSTQ on the fifth day on comparison to normal control. Treatment ferulic acid (15, 30 and 60 mg/kg) and donepezil significantly ($p<0.001$) reduced escape latency from the 2nd to 4th training days and significantly ($p<0.05$, $p<0.01$, $p<0.001$ and $p<0.001$ respectively) increased TSTQ by autistic pups on the 5th day relative to autistic control pups, indicating improvement of learning and memory (Table 1).

Table 1: Effect of ferulic acid on escape latency and time spent in target quadrant by rat pups using Morris water maze

Groups	Escape latency (sec)				Time spent in target quadrant, TSTQ (sec)
	Day 52 (1st day of training)	Day 53 (2nd day of training)	Day 54 (3rd day of training)	Day 55 (4th day of training)	
Normal control	63.66 ± 8.12	29.08 ± 2.77	18.54 ± 0.61	10.04 ± 0.69	66.66 ±3.32
Autistic control	90.45 ± 1.05##	76.5 ± 1.38###	57.62 ± 1.37###	34.5 ± 1.45###	38.83 ± 2.97###
Ferulic acid (15 mg/kg)	76.25 ± 4.54	48.25 ± 0.73***	31.33 ± 0.9***	14.75 ± 1.14***	54.66 ± 2.81*
Ferulic acid (30 mg/kg)	75.54 ± 3.13	40.66 ±1.19 ***	22.58 ± 0.51***	11.08 ± 0.49***	58.33 ± 5.11**

Ferulic acid (60 mg/kg)	78.29 ± 2.0	39.66 ± 4.57***	23.04 ± 1.59***	11.7 ± 0.56***	64.66 ± 6.61***
Donepezil (0.75 mg/kg)	66.66 ± 4.18***	29.87 ± 0.88***	17.29 ± 1.45***	13 ± 0.67***	74.66 ± 2.74***

Values are written as Mean ± SEM. n = 6 in each group. Analysis of data was performed using one-way analysis of variance followed by Tukey-Kramer test for multiple comparison.

Day 1 of trial- F (5,30) =4.52; p = 0.0034 Day 2 of trial- F (5,30) = 55.07; p<0.0001

Day 3 of trial- F (5,30) = 171.76; p<0.0001 Day 4 of trial- F (5,30) = 105; p<0.0001

TSTQ – F (5,30) = 11.28; p<0.0001

indicates p<0.01; ### indicates p<0.001 vs. normal control pups

* indicates p<0.05; ** indicates p<0.01; *** indicates p<0.001 vs. autistic control pups

3.8 Effect of ferulic acid on MAO-A activity in rat pups

Activity of MAO-A was found to be significantly elevated in autistic control pups compared to normal control pups (p<0.001). Treatment with ferulic acid (15, 30 and 60 mg/kg) exhibited significant decrease in activity of brain MAO-A of autistic pups relative to autistic control pups (p<0.001). (Figure 4-A).

3.9 Effect of ferulic acid on brain acetylcholinesterase activity in rat pups

Activity of acetylcholinesterase was observed to be significantly elevated (p<0.001) in autistic control pups compared with normal control pups. Treatment with ferulic acid (15, 30 and 60 mg/kg) as well as donepezil produced significant reduction in acetylcholinesterase activity (p<0.001) of autistic pups relative to autistic control pups (Figure 4-B).

3.10 Effect of ferulic acid on plasma corticosterone level

Plasma corticosterone levels, a key marker of stress, were found to be significantly elevated (p<0.001) in autistic rat pups compared with normal control. Administration of ferulic acid (30 and 60 mg/kg) and standard anti-depressant drug, fluoxetine (10 mg/kg) produced significant reduction in corticosterone concentrations (p<0.001) relative to vehicle-treated autistic rat pups. However, the lowest dose of ferulic acid (15 mg/kg) produced lesser significant (p<0.05) decrease of plasma corticosterone levels (Figure 4-C)

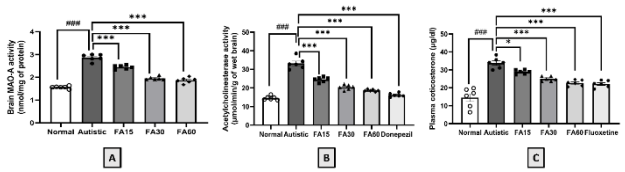

Figure 4. Effect of ferulic acid on (A) brain MAO-A activity: F (5,30) = 149.41 (B) brain acetylcholinesterase activity: F (5,30) = 10.6.02; (C) plasma corticosterone level: F (5,30) = 32.37 of rat pups. FA15/30/60 = ferulic acid 15/30/60 mg/kg. Here, values were written as Mean ± SEM. n = 6 in each group. Analysis of data was performed using one-way analysis of variance followed by Tukey-Kramer test for multiple comparison.

indicates p<0.001 vs. normal control pups

* indicates p<0.05, ** indicates p<0.01, *** indicates p<0.001 vs. autistic control pups

3.9 Effect of ferulic acid on MDA, nitrite, GSH and catalase in rat pups

Autistic control pups demonstrated significant oxidative stress, as evidenced by elevated brain malondialdehyde (MDA) and nitrite levels, along with significant decrease of reduced glutathione (GSH) concentrations and catalase activity on comparison to normal control. Treatment with ferulic acid at all the tested doses (15, 30 and 60 mg/kg) significantly lowered MDA and nitrite levels (p<0.001) and significantly increased brain GSH levels (p<0.001), whereas only medium and highest dose of ferulic acid (30 and 60 mg/kg) increased (p<0.001) catalase activity (Table 2).

Table 2: Effect of ferulic acid on oxidative stress parameters

Groups	Brain MDA (nmol/mg of protein)	Brain GSH (nmol/mg of protein)	Brain catalase (µmol of H_2O_2 decomposed/min/mg of protein)	Brain nitrite (µg/ml)
Normal pups	0.17 ± 0.005	12.63 ± 0.41	74.28 ± 3.49	27.96 ± 1.37
Autistic pups	0.58 ± 0.009###	4.27 ± 0.43###	36.01 ± 4.26###	75.22 ± 1.83###
Ferulic acid (15 mg/kg)	0.46 ± 0.009***	9.32 ± 0.60***	48.28 ± 1.89	60.04 ± 1.04***
Ferulic acid (30 mg/kg)	0.39 ± 0.010***	10.34 ± 0.30***	63.63 ± 4.53***	53.87 ± 1.89***
Ferulic acid (60 mg/kg)	0.30 ± 0.011***	10.89 ± 0.33***	77.55 ± 2.72***	35.96 ± 2.18***
Aripiprazole (1 mg/kg)	0.28 ± .013***	11.51 ± 0.39***	77.63 ± 3.62***	37.04 ± 1.24***

Here, values were written as Mean ± SEM. n = 6 in each group. Analysis of data was performed using one-way analysis of variance followed by Tukey-Kramer test for multiple comparison.

For MDA; F (5, 30) =204.4; p<0.0001 For GSH; F (5, 30) = 47.96; p<0.0001
For Catalase; F (5,3 0) = 23.93; p<0.0001 For Nitrite; F(5, 30)= 117.14; p<0.0001
p<0.001 vs. normal control pups
*** p<0.001 vs. autistic control pups

3.12 Effect of ferulic acid on brain histopathology

In normal control pups, the cerebellum exhibited a well-preserved histoarchitecture, with intact molecular layer (ML), Purkinje cell layer (PCL), and granular layer (GL). Prenatal exposure to sodium valproate (400 mg/kg; s.c) produced pronounced neuropathological alterations, evidenced by significant Purkinje cell necrosis, reduced Purkinje cell density, and depletion of granular cells. Administration medium (30 mg/kg) and highest dose (30 mg/kg) of ferulic acid to autistic pups conferred significant protective effect as reflected by restoration of cerebellar histology to near-normal architecture, with well-preserved layers and only mild necrosis, as indicated by a slight reduction in Purkinje cell count (Figure 5 A-E).

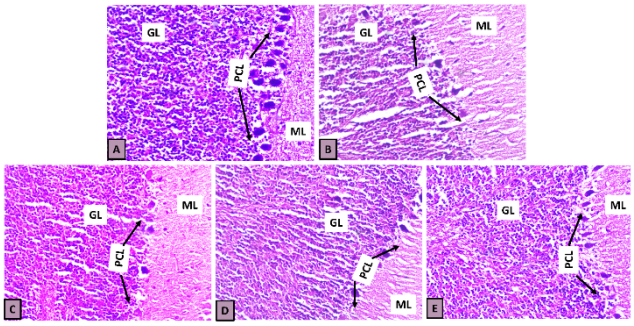

Figure 5. Effects of ferulic acid on histopathological changes in cerebellum part of brain of rat pups (H&E×400). A) Normal Control pups: Normal histological appearance of cerebellum depicting molecular layer (ML), Purkinje cell layer (PCL) and granular cell layer (GL). B) Autistic control pups: Marked necrosis characterized by the pyknotic changes in the nuclei of Purkinje cells (arrow heads), reduced number of Purkinje cells and depletion of cells in granular layer (less density in comparison to normal control pups).; C) Ferulic acid (15 mg/kg) in autistic pups: Microscopic changes similar to autistic control pups; D) Ferulic acid (30 mg/kg) in autistic pups: moderate improvement in necrosis characterized by less depletion of cells in granular layer as compared to autistic control pups, and pyknotic changes in the nuclei of a few Purkinje cells (arrow head). E) Ferulic acid (60 mg/kg) in autistic pups: Histological appearance of cerebellum of autistic pups comparable to normal control pups, having normal appearance of different layers except mild necrosis characterized by mild reduction in number of Purkinje cells.

4 DISCUSSION

The present study explored the therapeutic potential of ferulic acid in rat pups exposed prenatally to sodium valproate. Prenatal exposure to valproic acid is a well-established experimental model for autism spectrum disorder, inducing core autism-like behaviours in rodents. Valproic acid disrupts neurodevelopment via epigenetic mechanisms, notably histone deacetylase inhibition and defective histone acetylation, leading to impaired synaptic plasticity, altered gene expression, and abnormal cortical neurogenesis[59]. Autism was experimentally induced by a single subcutaneous injection of sodium valproate (400 mg/kg) to pregnant rats on gestational day 13. Offsprings from these female rats demonstrated characteristic autism-like features, including decreased body weight at birth, prolonged eye-opening time, and impairment of motor coordination, consistent with earlier findings[38, 39]. Only male pups were included for further experimentation, since male animals develop more autism-like alterations than females[28]. Similarly, in clinical reports, males have higher risk of developing the disorder[29, 30]. Effects on nociception, locomotor activity, depressive-like behavior, anxiety, learning and memory, and social behaviour of rat pups were examined. Autistic rat pups demonstrated significant hypoalgesia, hyperlocomotion, increased immobility in tail suspension test, learning and memory deficits, and impairment of social behavior.

Reduction in pain responsiveness has already been evidenced in autistic rat pups in previous studies[60, 61]. Notably, ferulic acid (60 mg/kg) significantly restored pain sensitivity in autistic rat pups in this study. Autistic pups also demonstrated significant elevation in locomotor activity, which is also supported by previous studies[38, 42]. All the tested doses of ferulic acid significantly attenuated hyperlocomotion in autistic rat pups in comparison to autistic control pups. Immobility period in tail suspension test, a parameter of depressive behaviour was significantly increased in autistic rat pups. The same effect has been reported earlier[62]. Ferulic acid significantly exhibited dose dependent effect and reduced depressive-like behaviour in autistic rat pups. Moreover, ferulic acid has also been reported to exert antidepressant effect in an earlier study[25]. The observed antidepressant effect of ferulic acid was comparable to fluoxetine (10 mg/kg), the standard antidepressant drug employed. Fluoxetine has also been reported to improve depression- like symptoms in autistic patients[63]. Anxious behaviour of autistic rat pups is exhibited by reduction of time spent along with number of entries in open arms of elevated zero maze, as also evidenced in previous studies[38, 64]. Ferulic acid (15, 30 and 60 mg/kg) administration to autistic rat pups significantly increased time and number of entries in open arms of elevated zero maze, indicating attenuation of anxiety- like behavior. Anti-anxiety activity of ferulic acid has also been reported earlier[23, 24]. The observed anxiolytic effect of ferulic acid was comparable to diazepam, the standard anxiolytic drug employed. In Morris water maze test, escape latency of autistic pups during training period of 4 days was remarkably increased along with decrease in TSTQ on 5th day (memory testing), denoting learning, and memory impairment. These effects in autistic pups have already been reported in previous studies[65, 66]. Ferulic acid significantly reversed learning and memory deficits in autistic pups, as reflected by reduced escape latency and increased TSTQ. Ferulic acid has also been shown to

possess memory enhancing effect in the literature[17, 18, 21]. The ferulic acid effects on improvement of learning and memory were observed to be comparable to donepezil, the standard memory enhancing drug employed. Donepezil has shown beneficial effect on memory and receptive language skills in autistic children[67, 68]. Impairment of social behavior is one of the core features evidenced in autistic pups[30] and autistic children[1]. Autistic pups exhibited decreased social interaction score, implicating impaired social behaviour in our study, which is also supported by the previous studies[38, 39]. Ferulic acid significantly increased social interaction of autistic rat pups when compared to autistic control pups.

Biochemical analyses were performed to find out the potential mechanisms underlying the observed effects. Autistic rat pups exhibited significant elevation of brain acetylcholinesterase activity, indicating reduced cholinergic transmission, which may contribute to cognitive impairment as evidenced by previous studies[69, 70]. All doses of ferulic acid significantly decreased acetylcholinesterase activity, thus potentially enhancing brain acetylcholine levels, leading to learning and memory improvement. Further, ferulic acid has been reported to inhibit acetylcholinesterase activity in an *in vivo* study[17] and *in vitro* study[18]. MAO-A activity was observed to be increased in autistic pups, which is also reported in the literature[71, 72]. By inhibition of MAO-A activity, ferulic acid might have elevated brain monoamine neurotransmitter dopamine, norepinephrine, and serotonin) concentrations, thereby alleviating depressive-like behaviour. Ferulic acid has also been reported to inhibit MAO-A activity in a previous study[25]. Oxidative stress contributes significantly in the pathophysiology of autism[73]. Prenatal sodium valproate exposure is reported to enhance generation of reactive oxygen and nitrogen species, which interfere with neuronal growth and maturation[39, 73]. In this study, autistic rat pups showed significantly increased MDA and nitrite levels, accompanied by decrease in GSH content and catalase activity in brain tissue, thus indicating rise in oxidative stress. The similar findings have been published in the past studies[26, 70, 72]. Ferulic acid administration at all doses significantly decreased oxidative stress markers along with restoration of antioxidant enzyme activities in this study. Ferulic acid has also shown antioxidant effect in previous studies[15, 74]. Plasma corticosterone, a key stress hormone, was reported to be elevated in autistic individuals[75]. It was also significantly increased in autistic rat pups in our study. These results were strengthened by the past studies[76, 77]. Administration of ferulic acid significantly lowered corticosterone level, thus attenuating stress in autistic pups. These results were supported by the previous studies conducted on ferulic acid[78, 79]

Histopathological studies were also carried out on cerebellar part of brain of rat pups. There were marked neuropathological alterations were observed in cerebellar part of brain of autistic pups, including degeneration and loss of Purkinje cells and depletion of granular layer neurons, thus affecting the motor coordination[80, 81]. In this study, autistic rat pups also exhibited pathological changes such as decreased count and pyknotic alterations in purkinje cells; and depletion of granular layer cells. These observations were also consistent with the previous studies[38, 82]. Ferulic acid (50 and 100 mg/kg) protected against these histopathological abnormalities, while restoring the normal cerebellar cytoarchitecture.

5.CONCLUSION

Based upon the above findings, we may conclude that ferulic acid exerted significant neuroprotective effect against sodium valproate-induced autism in rat pups, which might be mediated through multiple mechanisms, including improvement of cholinergic and monoaminergic neurotransmission, attenuation of oxidative stress, reduction of stress hormone levels, and preservation of cerebellar integrity.

CONFLICT OF INTEREST

The Authors declare that they have no conflict of interest.

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REFERENCE

1. Wang M, Zhang X, Zhong L, Zeng L, Li L, Yao P. Understanding autism: Causes, diagnosis, and advancing therapies. Brain Res Bull. 2025; 227:111411. doi: 10.1016/j.brainresbull.2025.111411.
2. Shaw KA, Williams S, Patrick ME, Valencia-Prado M, Durkin MS, Howerton EM et al. Prevalence and Early Identification of Autism Spectrum Disorder Among Children Aged 4 and 8 Years - Autism and Developmental Disabilities Monitoring Network, 16 Sites, United States, 2022. MMWR Surveill Summ. 2025;74(2):1-22. doi: 10.15585/mmwr.ss7402a1.
3. Rylaarsdam L and Guemez-Gamboa A. Genetic Causes and Modifiers of Autism Spectrum Disorder. Front Cell Neurosci. 2019;13: 385. doi: 10.3389/fncel.2019.00385.
4. Bougeard C, Picarel-Blanchot F, Schmid R, Campbell R, Buitelaar J. Prevalence of Autism Spectrum Disorder and Co-morbidities in Children and Adolescents: A Systematic Literature Review. Front Psychiatry. 2021;12: 744709. doi: 10.3389/fpsyt.2021.744709.
5. Jiang CC, Lin LS, Long S, Ke XY, Fukunaga K, Lu YM, Han F. Signalling pathways in autism spectrum disorder: mechanisms and therapeutic implications. Signal Transduct Target Ther. 2022;7(1):229. doi: 10.1038/s41392-022-01081-0.
6. Doyle CA, McDougle CJ. Pharmacologic treatments

for the behavioral symptoms associated with autism spectrum disorders across the lifespan. Dialogues Clin Neurosci. 2012; 14(3): 263-79. Doi: https://doi.org/10.31887/dcns.2012.14.3/cdoyle

7. McCracken JT, McGough J, Shah B, Cronin P, Hong D, Aman MG *et al*. Research Units on Pediatric Psychopharmacology Autism Network. Risperidone in children with autism and serious behavioral problems. N Engl J Med. 2002; 347(5): 314-21. Doi: https://doi.org/10.1056/nejmoa013171
8. Scahill L, McDougle CJ, Aman MG, Johnson C, Handen B, Bearss K *et al*. Effects of risperidone and parent training on adaptive functioning in children with pervasive developmental disorders and serious behavioral problems. J Am Acad Child Adolesc Psychiatry. 2012; 51(2): 136-46. Doi: https://doi.org/10.1016/j.jaac.2011.11.010
9. Urdaneta KE, Castillo MA, Montiel N, Semprún-Hernández N, Antonucci N, Siniscalco D. Autism spectrum disorders: potential neuro-psychopharmacotherapeutic plant-based drugs. Assay Drug Dev Technol. 2018; 16(8): 433-44. Doi: https://doi.org/10.1089/adt.2018.848
10. Singh K, Connors SL, Macklin EA, Smith KD, Fahey JW, Talalay P, Zimmerman AW. Sulforaphane treatment of autism spectrum disorder (ASD). Proc Natl Acad Sci U S A. 2014;111(43):15550-5. doi: 10.1073/pnas.1416940111.
11. Theoharides TC, Asadi S, Panagiotidou S. A case series of a luteolin formulation (NeuroProtek®) in children with autism spectrum disorders. Int J Immunopathol Pharmacol. 2012; 25(2): 317-323. Doi: https://doi.org/10.1177/039463201202500201
12. Tsilioni I, Taliou A, Francis K, Theoharides TC. Children with autism spectrum disorders, who improved with a luteolin-containing dietary formulation, show reduced serum levels of TNF and IL-6. Transl Psychiatry. 2015; 5(9): e647. Doi: https://doi.org/10.1038/tp.2015.142
13. Pretzsch CM, Voinescu B, Mendez MA, Wichers R, Ajram L, Ivin G *et al*. The effect of cannabidiol (CBD) on low-frequency activity and functional connectivity in the brain of adults with and without autism spectrum disorder (ASD). J Psychopharmacol. 2019; 33(9): 1141-8. Doi: https://doi.org/10.1177/0269881119858306
14. Aran A, Harel M, Cassuto H, Polyansky L, Schnapp A, Wattad N *et al*. Cannabinoid treatment for autism: a proof-of-concept randomized trial. Mol Autism. 2021; 12(1): 6. Doi: https://doi.org/10.1186/s13229-021-00420-2
15. Ren Z, Zhang R, Li Y, Li Y, Yang Z, Yang H. Ferulic acid exerts neuroprotective effects against cerebral ischemia/reperfusion-induced injury via antioxidant and anti-apoptotic mechanisms *in vitro* and *in vivo*. Int J Mol Med. 2017;40(5):1444-1456. doi: 10.3892/ijmm.2017.3127.
16. Liu YM, Shen JD, Xu LP, Li HB, Li YC, Yi LT. Ferulic acid inhibits neuro-inflammation in mice exposed to chronic unpredictable mild stress. Int Immunopharmacol. 2017;45:128-134. doi: 10.1016/j.intimp.2017.02.007.
17. Mahaman YAR, Huang F, Salissou MTM, Yacouba MBM, Wang JZ, Liu R, Zhang B, Li HL, Zhu F, Wang X. Ferulic Acid Improves Synaptic Plasticity and Cognitive Impairments by Alleviating the PP2B/DARPP-32/PP1 Axis-Mediated STEP Increase and Aβ Burden in Alzheimer's Disease. Neurotherapeutics. 2023;20(4):1081-1108. doi: 10.1007/s13311-023-01356-6.
18. Mugundhan V, Arthanari A, Parthasarathy PR. Protective Effect of Ferulic Acid on Acetylcholinesterase and Amyloid Beta Peptide Plaque Formation in Alzheimer's Disease: An *In vitro* Study. Cureus. 2024;16(2):e54103. doi: 10.7759/cureus.54103.
19. Ojha S, Javed H, Azimullah S, Abul Khair SB, Haque ME. Neuroprotective potential of ferulic acid in the rotenone model of Parkinson's disease. Drug Des Devel Ther. 2015;9:5499-510. doi: 10.2147/DDDT.S90616.
20. Abdelgawad MA, Gendy AM, Zaghlool SS, Elesawy WH, Ragab MF, Kotb El-Sayed MI et al. Ferulic acid mitigates 3-Nitropropionic acid-induced Huntington's disease via modulation of Nrf2/HO-1, TLR4/NF-κB, and SIRT1/p53 signaling pathways. Front Pharmacol. 2025;16:1678724. doi: 10.3389/fphar.2025.1678724.
21. Yang H, Qu Z, Zhang J, Huo L, Gao J, Gao W. Ferulic acid ameliorates memory impairment in d-galactose-induced aging mouse model. Int J Food Sci Nutr. 2016;67(7):806-17. doi: 10.1080/09637486.2016.1198890.
22. Hassanzadeh P, Arbabi E, Atyabi F, Dinarvand R. Ferulic acid exhibits antiepileptogenic effect and prevents oxidative stress and cognitive impairment in the kindling model of epilepsy. Life Sci. 2017;179:9-14. doi: 10.1016/j.lfs.2016.08.011.
23. Bhuia MS, Rokonuzzman M, Hossain MI, Ansari SA, Ansari IA, Islam T, Al Hasan MS, Mubarak MS, Islam MT. Anxiolytic-*like* Effects by *trans*-Ferulic Acid Possibly Occur through GABAergic Interaction Pathways. Pharmaceuticals (Basel). 2023;16(9):1271. doi: 10.3390/ph16091271.

24. Cetiner OP, Eken H, Altınok FA, Arslan R, Bektas N. Ferulic acid possesses anxiolytic activity: evidence for the involvement of serotonergic and noradrenergic systems. Neuroscience Letters. 2025. 869; 138416. https://doi.org/10.1016/j.neulet.2025.138416.
25. Chen J, Lin D, Zhang C, Li G, Zhang N, Ruan L et al. Antidepressant-like effects of ferulic acid: involvement of serotonergic and norepinergic systems. Metab Brain Dis. 2015;30(1):129-36. doi: 10.1007/s11011-014-9635-z.
26. Chatterjee D, Singh S. Neuroprotective effect of ferulic acid in valproic acid induced autism like behaviour in zebrafish via modulation of PI3K/AKT/mTOR pathway. Comp Biochem Physiol C Toxicol Pharmacol. 2025;299: 110347. doi: 10.1016/j.cbpc.2025.110347.
27. Forozan Y, Amini-Khoei H, Solati K, Sureda A, Lorigooin Z. Potential therapeutic effects of *Ferula gummosa* Boiss. Extract on Autistic-like behaviors in maternally separated mice: Role of NMDA receptor gene. Heliyon. 2025; 11(10): e42957
28. Schneider T, Roman A, Basta-Kaim A, Kubera M, Budziszewska B, Schneider K *et al*. Gender-specific behavioral and immunological alterations in an animal model of autism induced by prenatal exposure to valproic acid. Psychoneuroendocrinology. 2008; 33:728-40. 10.1016/j.psyneuen.2008.02.011.
29. Melancia F, Schiavi S, Servadio M, Cartocci V, Campolongo P, Palmery M, et al. Sex-specific autistic endophenotypes induced by prenatal exposure to valproic acid involve anandamide signalling. Br J Pharmacol. 2018; 175:3699-712. doi: 10.1111/bph.14435.
30. Loomes R, Hull L, Mandy WPL. What is the male-to-female ratio in autism spectrum disorder? a systematic review and meta-analysis. J Am Acad Child Adolesc Psychiatry. 2017; 56:466-74. doi: 10.1016/j.jaac.2017.03.013.
31. Kim J, Shin M, Seo T, Ji E, Baek S, Lee S *et al*. Treadmill exercise ameliorates motor disturbance through inhibition of apoptosis in the cerebellum of valproic acid-induced autistic rat pups. Molecular Medicine Reports. 2013; 8(2): 327-34. Doi: https://doi.org/10.3892/mmr.2013.1518
32. Senaphan K, Kukongviriyapan U, Sangartit W, Pakdeechote P, Pannangpetch P, Prachaney P et al. Ferulic Acid Alleviates Changes in a Rat Model of Metabolic Syndrome Induced by High-Carbohydrate, High-Fat Diet. Nutrients. 2015;7(8):6446-64. doi: 10.3390/nu7085283.
33. Scheggi S, Pelliccia T, Gambarana C, De Montis MG. Aripiprazole relieves motivational anhedonia in rats. J Affect Disord. 2018; 227:192-197. doi: 10.1016/j.jad.2017.10.032.
34. Jayakumar S, Raghunath G, Ilango S, Vijayakumar J, Vijayaraghavan R. Effect of Fluoxetine on the Hippocampus of Wistar Albino Rats in Cold Restraint Stress Model. J Clin Diagn Res. 2017;11 (6):AF01-AF06. doi: 10.7860/JCDR/2017/26958.9953.
35. Ghajarbeygi P, Hajhoseini A, Hosseini MS, Sharifan A. An *in vitro* and *in vivo* cholinesterase inhibitory activity of *Pistacia khinjuk* and *Allium sativum* essential oils. J Pharmacopuncture. 2019; 22(4): 231-8. Doi: https://doi.org/10.3831/kpi.2019.22.031
36. Gilhotra N, Dhingra D. GABAergic and nitriergic modulation by curcumin for its antianxiety-like activity in mice. Brain Research. 2010; 1352: 167-175. Doi: https://doi.org/10.1016/j.brainres.2010.07.007
37. Choi SH, Cho YS, Kim MJ, Lee CH, Seong HH, Baek SH, et al. Studies on the induction of pregnancy and the number of fetuses during pregnancy in rats. J Anim Reprod Biotech. 2020; 35:232-8. Doi: https://doi.org/10.12750/JARB.35.3.232
38. Sandhya T, Sowjanya J, Veeresh B. *Bacopa monniera* (L.) Wettst ameliorates behavioral alterations and oxidative markers in sodium valproate induced autism in rats. Neurochem Res. 2012; 37:1121-31. doi: 10.1007/s11064-012-0717-1.
39. Mirza R, Sharma B. Beneficial effects of pioglitazone, a selective peroxisome proliferator-activated receptor-γ agonist in prenatal valproic acid-induced behavioral and biochemical autistic like features in Wistar rats. Int J Dev Neurosci. 2019; 76: 6-16. doi: 10.1016/j.ijdevneu.2019.05.006.
40. Ruhela RK, Soni S, Sarma P, Prakash A, Medhi B. Negative geotaxis: An early age behavioral hallmark to VPA rat model of autism. Ann Neurosci. 2019; 26: 25-31. doi: 10.5214/ans.0972.7531.260106
41. Ramabadran K, Bansinath M, Turndorf H, Puig MM. Tail immersion test for the evaluation of a nociceptive reaction in mice. Methodological considerations. J Pharmacol Methods. 1989; 21:21-31. doi: 10.1016/0160-5402(89)90019-3.
42. Luhach K, Kulkarni GT, Singh VP, Sharma B. Cilostazol attenuated prenatal valproic acid-induced behavioural and biochemical deficits in a rat model of autism spectrum disorder. J Pharm Pharmacol. 2021; 73:1460-9. doi: 10.1093/jpp/rgab115.
43. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology (Berl). 1985; 85:367-70. doi: 10.1007/BF00428203.
44. Dhingra D, Bansal S. Antidepressant-like activity of

plumbagin in unstressed and stressed mice. Pharmacol Rep. 2015; 67:1024-32. Doi: 10.1016/j.pharep.2015.03.001.

45. Kulkarni SK, Singh K, Bishnoi M. Elevated zero maze: a paradigm to evaluate antianxiety effects of drugs. Methods Find Exp Clin Pharmacol. 2007; 29:343-8. doi: 10.1358/mf.2007.29.5.1117557.
46. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat, J Neurosci Methods.1984; 11:47-60. doi: 10.1016/0165-0270(84)90007-4.
47. Rahi S, Gupta R, Sharma A, Mehan S. Smo-Shh signaling activator purmorphamine ameliorates neurobehavioral, molecular, and morphological alterations in an intracerebroventricular propionic acid-induced experimental model of autism. *Hum Exp Toxicol*. 2021;40 (11):1880-1898. doi:10.1177/09603271211013456
48. Thornton AM, Humphrey RM, Kerr DM, Finn DP, Roche M. Increasing Endocannabinoid Tone Alters Anxiety-Like and Stress Coping Behaviour in Female Rats Prenatally Exposed to Valproic Acid. Molecules. 2021; 26(12):3720. doi: 10.3390/molecules26123720.
49. Dhingra D, Soni K. Behavioral and biochemical evidences for nootropic activity of boldine in young and aged mice. Biomed Pharmacother. 2018;97: 895-904. doi: 10.1016/j.biopha.2017.11.011.
50. Schurr A, Livne A. Differential inhibition of mitochondrial monoamine oxidase from brain by hashish components. Biochem Pharmacol. 1976; 25:1201-3. Doi: 10.1016/0006-2952(76)90369-5.
51. Wills E. Mechanisms of lipid peroxide formation in animal tissues. Biochemical journal. 1966; 99(3): 667. doi: 10.1042/bj0990667.
52. Ellman GL. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 1959; 82:70–77. doi: 10.1016/0003-9861(59)90090-6.
53. Claiborne A. Catalase activity. In: Greenwald RA, editor. Handbook of methods for oxygen radical research. Boca Raton: CRC; 1985. 283–4.
54. Green L, Wagner D, Glogowski J, Skipper P, Wishnok J, Tannenbaum S. Analysis of nitrate, nitrite, and nitrate in biological fluids, Anal. Biochem. 1982; 126:131–8. doi: 10.1016/0003-2697(82)90118-x.
55. Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; 7:88-95. doi: 10.1016/0006-2952(61)90145-9.
56. Bartos J, Pesez M. Colorimetric and fluorimetric determination of steroids. Pure Appl Chem. 1979; 51:2157–69. Doi: https://doi.org/10.1016/0039-9140(80)80183-4
57. Valecha R, Dhingra D. Behavioral and Biochemical Evidences for Antidepressant-Like Activity of *Celastrus Paniculatus* Seed Oil in Mice. Basic Clin Neurosci. 2016; 7: 49-56. Doi: 10.1016/j.pharep.2013.06.001.
58. D'Mello AM, Stoodley CJ. Cerebro-cerebellar circuits in autism spectrum disorder. Front Neurosci. 2015; 9:408. Doi: 10.3389/fnins.2015.00408.
59. Yang J, Li X, Tan J, Zhou P, Hu L, Chen J, Li T, Liu Y, Chen L. Prenatal Exposure To Valproic Acid Induces Increased Autism-Like Behaviors and Impairment of Learning and Memory Functions in Rat Offspring by Upregulating ADAM10 Expression. Neurochem Res. 2025;50(3):146. doi: 10.1007/s11064-025-04398-8.
60. Juybari KB, Sepehri G, Meymandi MS, Vakili Shahrbabaki SS, Moslemizadeh A, Saeedi N *et al*. Sex dependent alterations of resveratrol on social behaviors and nociceptive reactivity in VPA-induced autistic-like model in rats. Neurotoxicol Teratol. 2020; 81:106905. Doi: 10.1016/j.ntt.2020.106905.
61. Seyedinia SA, Tarahomi P, Abbarin D, Sedaghat K, Rashidy-Pour A, Yaribeygi H *et al.* Saffron and crocin ameliorate prenatal valproic acid-induced autistic-like behaviors and brain oxidative stress in the male offspring rats. Metab Brain Dis. 2023; 38: 2231-41. doi: 10.1007/s11011-023-01275-7
62. Seiffe A, Ramírez MF, Sempé L, Depino AM. Juvenile handling rescues autism-related effects of prenatal exposure to valproic acid. Sci Rep. 2022; 12: 7174. doi: 10.1038/s41598-022-11269-7.
63. Lucchelli JP, Bertschy G. Low-Dose Fluoxetine in Four Children with Autistic Spectrum Disorder Improves Self-Injurious Behavior, ADHD-Like Symptoms, and Irritability. Case Rep Psychiatry. 2018; 2018: 6278501. doi: 10.1155/2018/6278501
64. Pobbe RL, Defensor EB, Pearson BL, Bolivar VJ, Blanchard DC, Blanchard RJ. General and social anxiety in the BTBR T+ tf/J mouse strain. Behav Brain Res. 2011; 216: 446-51. doi: 10.1016/j.bbr.2010.08.039.
65. Wu H, Wang X, Gao J, Liang S, Hao Y, Sun C *et al*. Fingolimod (FTY720) attenuates social deficits, learning and memory impairments, neuronal loss and neuroinflammation in the rat model of autism. Life Sci. 2017; 173:43-54. doi: 10.1016/j.lfs.2017.01.012.
66. Sharma AR, Batra G, Dhir N, Jain A, Modi T, Saini L *et al*. "Comparative evaluation of different chemical agents induced Autism Spectrum Disorder in experimental Wistar rats". Behav Brain Res. 2024; 458:114728. doi: 10.1016/j.bbr.2023.114728.
67. Srivastava RK, Agarwal M, Pundhir A. Role of

donepezil in autism: its conduciveness in psychopharmacotherapy. Case Rep Psychiatry. 2011; 2011:563204. doi: 10.1155/2011/563204

68. Gabis LV, Ben-Hur R, Shefer S, Jokel A, Shalom DB. Improvement of Language in Children with Autism with Combined Donepezil and Choline Treatment. J Mol Neurosci. 2019; 69:224-34. doi: 10.1007/s12031-019-01351-7.
69. Al-Amin MM, Rahman MM, Khan FR, Zaman F, Mahmud Reza H. Astaxanthin improves behavioral disorder and oxidative stress in prenatal valproic acid-induced mice model of autism. Behav Brain Res. 2015; 286:112-21. doi: 10.1016/j.bbr.2015.02.041.
70. Nakhal MM, Jayaprakash P, Aburuz S, Sadek B, Akour A. Canagliflozin ameliorates oxidative stress and autistic-like features in valproic-acid-induced autism in rats: comparison with aripiprazole action. Pharmaceuticals (Basel). 2023; 16:769. doi: 10.3390/ph16050769.
71. Wu JB, Shih JC. Valproic acid induces monoamine oxidase A via Akt/forkhead box O1 activation. Mol Pharmacol. 2011; 80:714-23. doi: 10.1124/mol.111.072744.
72. Salem, Fatma El Zahraa & Abdel-Gaber, Rewaida & Al-Megrin, Wafa & El-Khadragy, Manal. Therapeutic potency of *Camellia sinensis* extract on neurochemical and oxidative changes correlates to autistic disorder in rat pups model. Indian J Anim Res. 2023; 57:1652-61. Doi: 10.18805/IJAR.BF-1666
73. Gąssowska-Dobrowolska M, Cieślik M, Czapski GA, Jęśko H, Frontczak-Baniewicz M, Gewartowska M *et al*. Prenatal exposure to valproic acid affects microglia and synaptic ultrastructure in a brain-region-specific manner in young-adult male rats: relevance to autism spectrum disorders. Int J Mol Sci. 2020; 21:3576. doi: 10.3390/ijms21103576.
74. Itagaki S, Kurokawa T, Nakata C, Saito Y, Oikawa S, Kobayashi M, Hirano T, Iseki K. *In vitro* and *in vivo* antioxidant properties of ferulic acid: a comparative study with other natural oxidation inhibitors. Food Chemistry. 2009;114(2):466-71. Doi: https://doi.org/10.1016/j.foodchem.2008.09.073
75. Spratt EG, Nicholas JS, Brady KT, Carpenter LA, Hatcher CR, Meekins KA *et al*. Enhanced cortisol response to stress in children in autism. J Autism Dev Disord. 2012; 42:75-81. doi: 10.1007/s10803-011-1214-0
76. Frye CA, Llaneza DC. Corticosteroid and neurosteroid dysregulation in an animal model of autism, BTBR mice. Physiol Behav. 2010; 100: 264-7. doi: 10.1016/j.physbeh.2010.03.005
77. Kazlauskas N, Seiffe A, Campolongo M, Zappala C, Depino AM. Sex-specific effects of prenatal valproic acid exposure on sociability and neuroinflammation: Relevance for susceptibility and resilience in autism. Psychoneuroendocrinology. 2019; 110:104441. doi: 10.1016/j.psyneuen.2019.104441.
78. Zheng X, Cheng Y, Chen Y, Yue Y, Li Y, Xia S, Li Y, Deng H, Zhang J, Cao Y. Ferulic Acid Improves Depressive-Like Behavior in Prenatally-Stressed Offspring Rats via Anti-Inflammatory Activity and HPA Axis. Int J Mol Sci. 2019;20(3):493. doi: 10.3390/ijms20030493.
79. Sasaki K, Iwata N, Ferdousi F, Isoda H. Antidepressant-Like Effect of Ferulic Acid via Promotion of Energy Metabolism Activity. Mol Nutr Food Res. 2019;63(19):e1900327. doi: 10.1002/mnfr.201900327.
80. Cho HS, Kim TW, Ji ES, Park HS, Shin MS, Baek SS. Treadmill exercise ameliorates motor dysfunction through inhibition of Purkinje cell loss in cerebellum of valproic acid-induced autistic rats. J Exerc Rehabil. 2016; 12:293-8. doi: 10.12965/jer.1632696.348.
81. Kern JK & Jones AM. Evidence of Toxicity, Oxidative Stress, and Neuronal Insult in Autism. Journal of Toxicology and Environmental Health, Part B. 2006; 9:485–99. doi: 10.1080/10937400600882079.
82. Morakotsriwan N, Wattanathorn J, Kirisattayakul W, Chaisiwamongkol K. Autistic-like behaviors, oxidative stress status, and histopathological changes in cerebellum of valproic acid rat model of autism are improved by the combined extract of Purple rice and Silkworm pupae. Oxid Med Cell Longev. 2016; 2016:3206561. doi: 10.1155/2016/3206561.

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