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Neuroprotective Effect of Resveratrol on Valproic Acid Induced Oxidative Stress Autism in Swiss Albino Mice

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ABSTRACT

The present research work is aimed to investigate the anti-oxidant/neuroprotective role of Resveratrol in reversing the valproic acid induced autism in postnatal swiss albino mice. Separate 13 day old/ Post natal day (PND) 13 swiss albino mice of either sex into 5 groups, each group consists of six mice of either sex, groups namely Group I - Control, Group II - Resveratrol, Group III - Negative control, Group IV & V - Resveratrol treatment groups. On PND 14 administer single dose of Valproic acid (VPA) or Sodium Valproate (400 mg/kg, subcutaneously) to III, IV & V groups to induce autism. Treatment is given in two doses 10 mg/kg, intraperitoneally (*i.p*) as Low dose and 40 mg/kg, *i.p* as High dose from the 13th day to the end of study. Assessment of autism is done by different behavioral screening methods during PND 14 to 40. Treatment with resveratrol significantly decline the autism symptoms compared with negative control. At the end of study on PND 41 all the animals were sacrificed to assess the biochemical estimations like Anticholinesterase enzyme, Total Protein, antioxidant enzyme (Catalase, Superoxide and Glutathione) activity and cerebellar histopathological examination. Treatment with Resveratrol has shown a significant beneficial difference on behavioral alterations, oxidative markers, neurotransmitters, and restoration of the altered purkinje cells of autism. This research work we conclude that resvertrol have a potent anti-oxidant, neuroprotective, anxiolytic, learning & memory enhancing agent against valproic acid induced autism.

Keywords: Valproic acid, Oxidative stress, Autism, Resveratrol, Neuroprotective effect.

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INTRODUCTION

Autism is a neurodevelopmental disorder characterized by pervasive impairments in social interactions, verbal and nonverbal communications, repetitive patterns of behaviors, and lack of interests in daily activities. [1] Autism is a lifelong persistence disorder, typically

manifests in children by the age of three; prevalence rate is more in males than females. [2] Autism majorly affects the areas of communication, socialization, cognition, imagination and intuitive thought. Autism is a behaviorally defined disorder and is classified under the pervasive developmental disorders (PDDs). [3] Pervasive developmental disorders include Autism, Asperger's syndrome, Rett's disorder, Childhood disintegrative disorder. [4] Fetal developmental stages are crucial for the autism occurrence; it's majorly linked to abnormal biology and chemistry in the fetus brain. Oxidative stress during developmental stages majorly affects the functions of brain and disturbing the time of brain development. [5-6] Environmental factors have major contribution in the development of autism, which includes mercury, lead, measles, rubella virus, retinoic acid, maternal thalidomide, valproic acid. These teratogens enter the fetus brain and disturb the brain development by oxidative stress. [7-8]

Valproic acid (VPA) is a widely used antiepileptic drug and is highly effective in the treatment of myoclonic, atonic absence, and generalized seizures in children and adults. [9] VPA also has anti-manic properties that are widely used to treat epilepsy and bipolar disorders as well as migraines and generalized mood disorder. [10] Apart from medical uses, Valproic acid is a potent teratogen to the fetus, during post natal days which increases the risk of spina bifida aperta, as well as cardiac malformations, cleft palate, and limb defects. [11] Early exposure to valproic acid, during prenatal or postnatal period can serve as triggering factor for autism. [12-14]

Resveratrol (3, 5, 4`-trihydroxystilbene) is a naturally occurring non-flavonoid polyphenol belonging to the phytoalexin family. Resveratrol is found in peanuts, skin of grapes, blue berries and senna. Resveratrol haves cardio protection, anticancer, antidiabetic, antidepression, alzheimers and neuroprotective effects against diabetes-induced oxidative damage. [15] Resveratrol exhibits antioxidant activities through inhibiting quinone reductase 2, which in turn up-regulates the expression of cellular antioxidant and detoxification of enzymes to improve cellular resistance to oxidative stress. [16-17]

Apart from the benefits in various neurological diseases, in our research work aimed to study the role of anti-oxidant activity of resveratrol in autism. Valproic acid exposed swiss albino mice showed behavioral deficits such as social impairments and neurochemical alterations, administration of resveratrol successfully attenuated the valproic acid affects, proved its therapeutic effects.

MATERIALS AND METHODS

Drugs and chemicals

Resveratrol was gift sample from Samilabs, Bangalore. Sodium Valproate Injection (Brand ENCORATE, Sun Pharmaceuticals Ltd. India.) was purchased from pharma store. DTNB (5, 5'-dithiobis (2-nitrobenzoicacid)), Eserine, Acetylthiocholine Iodide, Reduced Glutathione were purchased from Sigma Aldrich, USA. All other reagents of Analytical grade are from S. D Fine chemicals Ltd, India.

Experimental animals

Swiss albino mice pups, weighing 5-15 g, at the age 2 weeks were used in the study. They were placed along with the mother, which was maintained on standard laboratory pellet chow diet, Provimi limited (India), provided water ad libitum and were kept under standard conditions at 23-25°C, 35 to 60% humidity, 12 h light /dark cycle. The mice were acclimatized to the laboratory conditions for a week prior to experiment. The experimental protocol was duly approved by Institutional Animal Ethics Committee (IAEC) and care of the animals was carried out as per the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA) (Protocol. No: I/IAEC/LCP/018/2014/SM-30♂ or ♀).

Experimental design

Female mice were checked daily and the day of birth was recorded as postnatal day (PND) 0. On the PND 13 animals were randomly divided into five groups of each six mice (n=6). All the animals except the vehicle control group and group treated with resveratrol alone were injected subcutaneously with Sodium Valproate (400 mg/kg) on PND 14. [12-14] Group I served as Vehicle control receives 20% Ethanol diluted using saline through intraperitoneal route (*i.p.*). Purpose of the use of ethanol is to increase the solubility of resveratrol. Group II receive only Resveratrol 40 mg/kg dissolved in vehicle through *i.p.* from PND 13 to 40. Group-III served as VPA group receives only injection with sodium valproate (400 mg/kg) subcutaneously (*s.c.*) on PND 14. Group-IV received sodium valproate on PND 14 followed by resveratrol 10 mg/kg dissolved in vehicle *i.p.* daily from PND 13 to 40 and was considered as low dose treated group. Group-V received sodium valproate on PND 14 followed by resveratrol 40 mg/kg (*i.p.*) daily from PND 13 to 40 and was considered as high dose treated group.

Mice pups were subjected to behavioral testing to assess motor co-ordination, nociceptive response, locomotion, anxiety, and cognition on various postnatal days up to postnatal day 40. At the end of behavioural testing, animals were sacrificed; brain was isolated for biochemical estimations and histopathological examination.

Behavioural Studies

Negative geotaxis

Negative geotropism was tested on postnatal days 14–19 by placing the mouse facing downward along a 45° incline in a temperature controlled environment. Latency to turn 180° such that the head was facing upward along the incline was recorded with a maximum of 30 sec for each trial. [18]

Motor coordination test

This utilizes a rotarod maintained at a speed of 40 rotations per minute (rpm). On PND 24, 25 and 26 mice

were placed individually on the rotating rod and the time taken by each animal to maintain its balance on the rod over a 5-min period was recorded. [19]

Locomotor activity using actophotometer

Spontaneous locomotor activity was measured daily on PND 34-37 in an activity cage having dimensions of 39×28×26 cm. The breakage of photo beams was monitored with infrared sensors and automatically recorded for 5 min. [20]

Nociception test

On PND 37, 38 and 39 mice were placed individually on a hot plate (55.0±0.3°C) and latency of the first hind-paw response was recorded. The hind-paw response was defined as either a foot shake or a paw lick. Cut off time of 30 sec was maintained. [21]

Elevated plus-maze test for anxiety

The elevated plus-maze consisted of two open arms (25 cm×5 cm) and two enclosed arms of the same size, with 15 cm high opaque walls. The maze elevated to a height of 55 cm above the floor. Each mouse was placed on PND 40 in the central square of the maze (5 cm×5 cm), facing one of the closed arms. Mouse behavior was recorded during a 10-min test period on PND 40. The number of entries into the open/closed arms and time spent in each of them was utilized for data analysis. [22]

Open field habituation

The exploratory behavior of the mice was evaluated by open field habituation task method. Mice was placed in a 40 cm×50 cm×60 cm open field whose brown linoleum floor was divided into 12 equal squares by white lines and left to explore it freely for 5 minutes on PND 40. The number of line crossings and head dippings were counted. [23]

Learning and memory test using morris water maze (MWM)

Morris water-maze test was employed to assess learning and memory of the animals. [24-25] It consists of large circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water maintained at 28±1°C). The water was made opaque with white colored non-toxic dye. A starting point was determined randomly from four equally placed quadrants. A submerged platform (10×10 cm), painted in white was placed inside the target quadrants of this pool, 1 cm below surface of water. The position of the hidden platform remained same throughout the experiment and the room was illuminated and an extra maze cues were present. If the animal did not reach the platform in 60 s, animal was gently guided to and placed on the platform. During the intervals all animals rested atop the platform until the next trial began. The animals were given three trials and the latency time to reach the platform was recorded in each trial and the mean was calculated. A significant decrease in latency time compared with first session was considered as successful learning. [26]

Biochemical parameters

Mice brains were isolated washed with ice-cold phosphate buffer to remove blood and homogenized with 10% phosphate buffer saline solution. The homogenate and the resultant supernatant were used for further biochemical estimations, such as AChE and antioxidants.

Estimation of acetyl cholinesterase (AChE) activity

The AChE activity was measured in brain tissue by the reaction of thiocholine with dithiobisnitrobenzoate ions. The rate of formation of thiocholine from acetylcholine iodide in the presence of brain cholinesterase was measured using a spectrophotometer (Shimadzu 1800) at a wavelength of 412 nm. [27-28]

Estimation of reduced glutathione (GSH)

GSH was determined by its reaction with 5, 5-dithiobis (2-nitrobenzoic acid) (Ellman's reagent) to yield a yellow chromophore which was measured spectrophotometrically. The brain homogenate was mixed with an equal amount of 10% trichloroacetic acid (TCA) and centrifuged (Remi cold centrifuge) at 2000×g for 10min at 4°C. To 0.1 ml of processed tissue sample, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5, 5-dithiobis(2-nitrobenzoic acid) (DTNB) and 0.4 ml of double-distilled water were added and the mixture was shaken. The absorbance was read at 412 nm within 15 min. [29]

Estimation of catalase

The rate of decomposition of H_2O_2 (Hydrogen Peroxide) to water and molecular oxygen is proportional to the activity of catalase. The sample containing catalase is incubated in the presence of a known concentration of H_2O_2. After incubation for exactly one minute, the reaction is stopped with ammonium molybdate. The amount of H_2O_2 remaining in the reaction is then determined by the oxidative coupling reaction between molybdate and H_2O_2. [30]

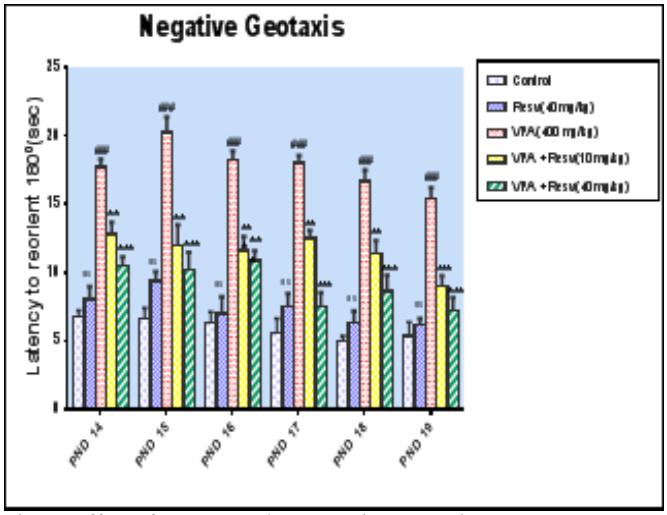

Fig. 1. E ffect of Resveratrol on negative geotaxis

Values are expressed as mean ± SEM of n=6 animals. Superscript letters represents the statistical significance done by ANOVA, followed by Tukey's tests. ###P<0.001 Compared with Vehicle; **P<0.01, ***P<0.001 Compared with Negative Control, ns=Not significant Compared with Vehicle. PND 14: F(4, 20)=31.65, PND 15: F(4,21)=15.67, PND 16: F(4,20)=24.97, PND 17: F(4,21)=33.25, PND 18: F(4,23)=32.31, PND 19: F(4,23)=21.73.

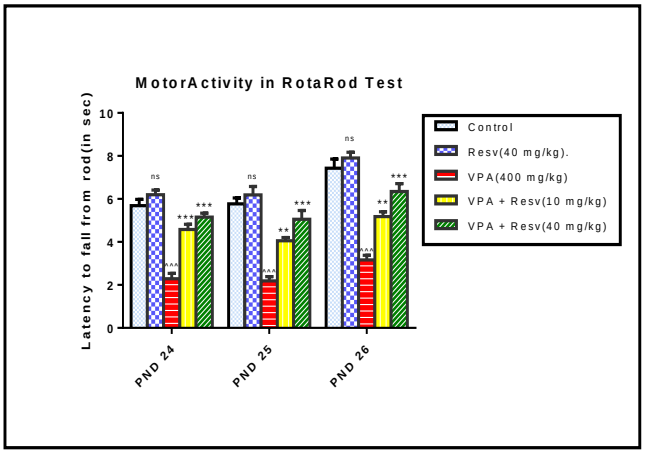

Fig. 2. Effect of Resveratrol on motor coordination.
Values are expressed as mean ± SEM of n=6 animals. Superscript letters represents the statistical significance done by ANOVA, followed by Tukey's tests. ^^^P<0.001 Compared with Vehicle; **P<0.01, ***P<0.001 Compared with Negative Control, ns=Not significant Compared with Vehicle. PND 24: F(4,20)=39.69, PND 25: F(4,20)=27.31, PND 26: F(4,20)=36.86.

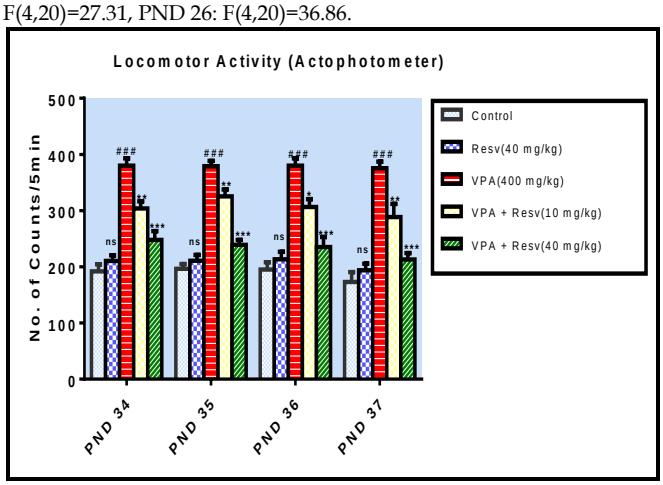

Fig. 3. Effect of Resveratrol on locomotor activity
Values are expressed as mean ± SEM of n=6 animals. Superscript letters represents the statistical significance done by ANOVA, followed by Tukey's tests. ###P<0.001 Compared with Vehicle; *P<0.05, **P<0.01, ***P<0.001 Compared with Negative Control, ns=Not significant Compared with Vehicle. PND 34: F(4,19)=37.70, PND 35: F(4,21)=63.16, PND 36: F(4,20)=26.26, PND 37: F(4,22)=31.13.

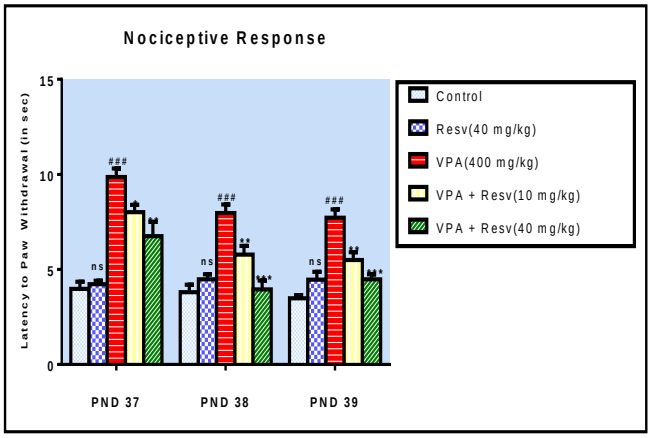

Fig. 4. Effect of Resveratrol on nociceptive response
Values are expressed as mean ± SEM of n=6 animals. Superscript letters represents the statistical significance done by ANOVA, followed by Tukey's tests. ###P<0.001 Compared with Vehicle; *P<0.05, **P<0.01, ***P<0.001 Compared with Negative Control, ns=Not significant Compared with Vehicle. PND 37: F(4,23)=20.22, PND 38: F(4,22)=24.80, PND 39: F(4,21)=17.96.

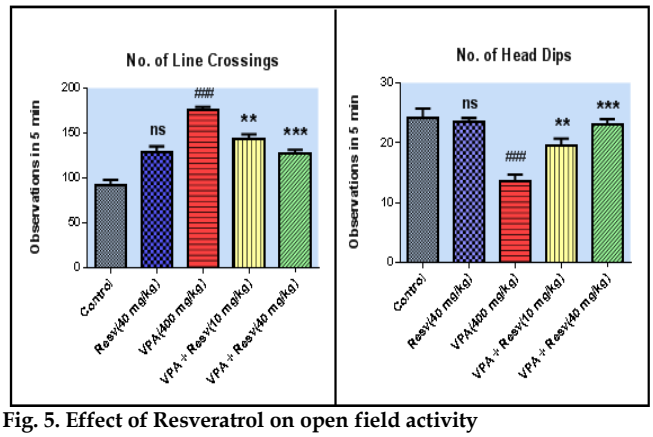

Fig. 5. Effect of Resveratrol on open field activity
Values are expressed as mean ± SEM of n=6 animals. Superscript letters represents the statistical significance done by ANOVA, followed by Tukey's tests. ###P<0.001 Compared with Vehicle; *P<0.05, **P<0.01, ***P<0.001 Compared with Negative Control, ns=Not significant Compared with Vehicle. No. of line crossing: F (4, 16)=30.57, No. of head dips: F(4,20)=15.87.

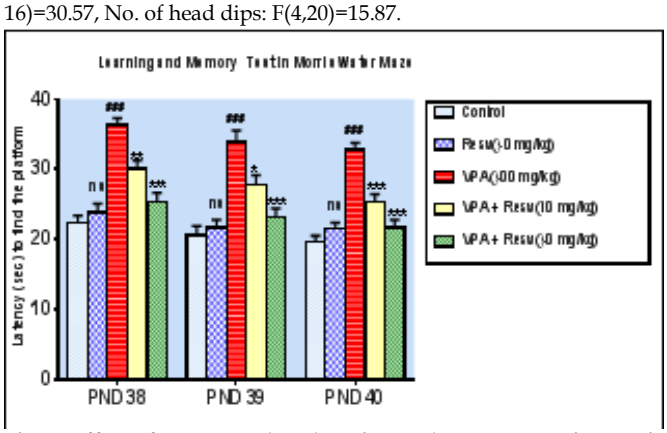

Fig. 6. Effect of Resveratrol on learning and memory test in morris water maze
Values are expressed as mean ± SEM of n=6 animals. Superscript letters represents the statistical significance done by ANOVA, followed by Tukey's tests. ###P<0.001 Compared with Vehicle; *P<0.05, **P<0.01, ***P<0.001 Compared with Negative Control, ns=Not significant Compared with Vehicle. PND 38: F(4,25)=24.10, PND 39: F(4,25)=16.46, PND 40: F(4,25)=27.59.

Estimation of superoxide dismutase

Superoxide dismutase (SOD) activity was determined by pyrogallol oxidation method. One unit SOD activity is defined as the amount of enzyme that inhibits the rate of auto-oxidation of pyrogallol by 50%. The reaction is initiated by adding pyrogallol and the change in optical density was recorded at 420 nm. [31]

Histopathology

Animals were sacrificed and brains were isolated immediately and placed in 10% neutral formalin solution, processed and embedded in paraffin. Sagittal sections of cerebellum (5µm thick) were stained with haemotoxylin and eosin (H&E) and analyzed using a light microscope for changes in Purkinje cell layer and photographs of sections were taken using camera.

Statistical analysis

The data was statistically analysed using one-way ANOVA followed by Tukey's tests and expressed as mean ± standard error of mean. P<0.05 was considered to be statistically significant. The statistical analysis was carried out with Graph pad prism 5.0 software.

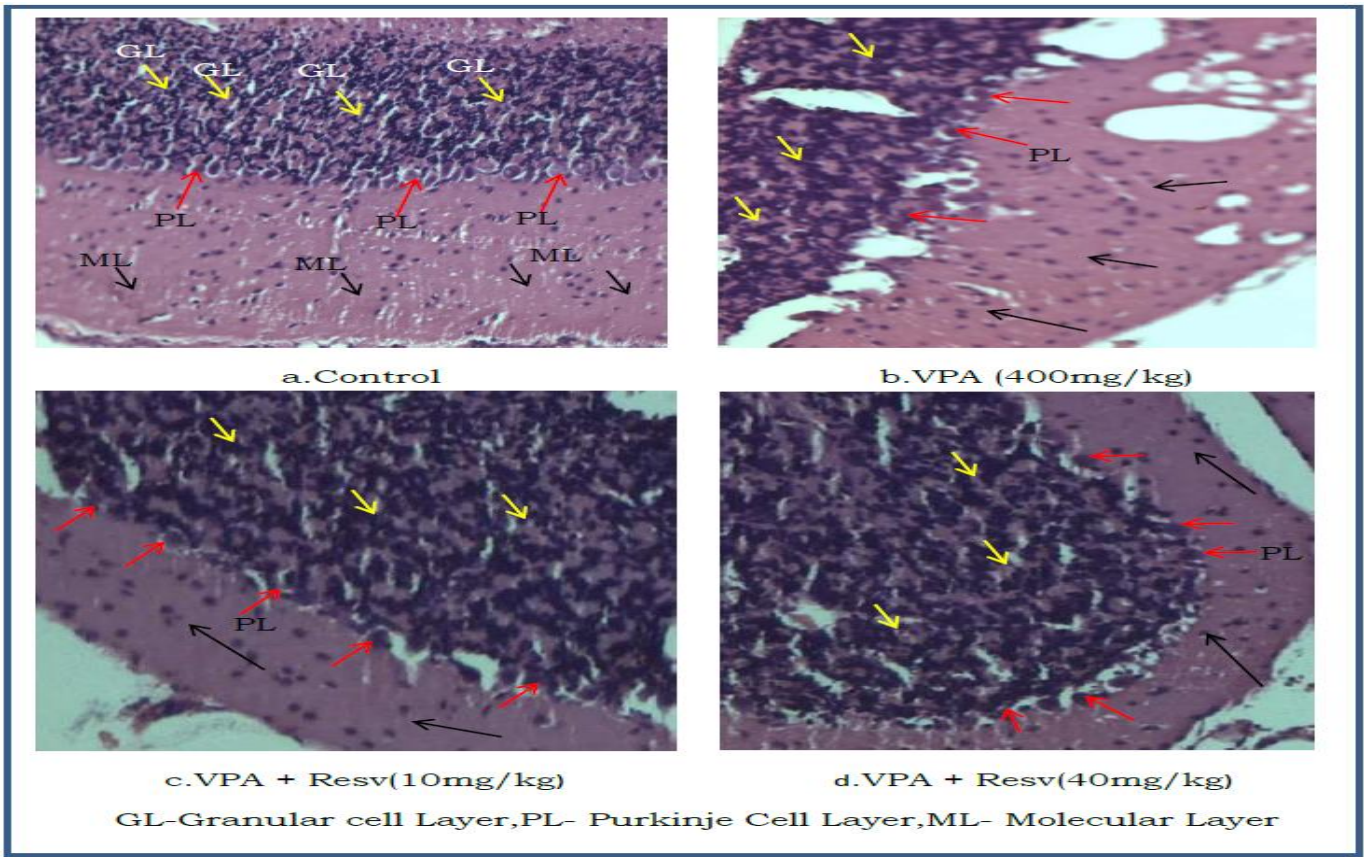

Fig. 7: Effect of Resveratrol on histopathology

Table 1: Effect of resveratrol on anxiety on elevated plus maze

Groups	No. of entries into open arms	Time spent in open arms (sec)	No. of entries into enclosed arms	Time spent in enclosed arms (sec)
Group I (Vehicle)	10.6 ± 0.51	189 ± 3.293	6.67 ± 0.422	104 ± 3.293
Group II (Resv 40mg/kg)	9.5 ± 1.041^{ns}	191.8 ± 4.466^{ns}	5 ± 0.365^{ns}	101.2 ± 4.466^{ns}
Group III (VPA 400mg/kg)	5.2 ± 0.374$^{###}$	92.59 ± 3.188$^{###}$	11.17 ± 0.601$^{###}$	200.4 ± 3.189$^{###}$
Group IV Low Dose (VPA +Resv 10 mg/kg)	8 ± 0.707*	138.9 ± 4.637***	7.83 ± 0.543***	154.1 ± 4.637***
Group V High Dose (VPA +Resv 40 mg/kg)	10 ± 0.707***	178.7 ± 7.193***	7.17 ± 0.307***	114.3 ± 7.193***

Values are expressed as mean ± SEM of n=6 animals. Superscript letters represents the statistical significance done by ANOVA, followed by Tukey's tests. ###P<0.001 Compared with Vehicle; *P<0.05, ***P<0.001 Compared with Negative Control, ^{ns}=Not significant Compared with Vehicle No. of open arm entries: F(4,19)=10.73, Time spent in open arm: F(4,18)=74.23, No. of closed entries: F(4,25)=24.25, Time spent in closed arms: F(4,18)=74.23.

Table 2: Effect of resveratrol on biochemical parameters

Groups	Total protein (mg/ml)	AChE (µM/min/mg tissue)	Glutathione (U/mg Protein)	Catalase (U/mg Protein)	SOD (U/mg Protein)
Group I (Vehicle)	25.62 ± 0.947	10.51 ± 0.673	8.56 ± 0.419	1.35 ± 0.038	15.45 ± 0.651
Group II (Resv 40 mg/kg)	22.43 ± 1.146^{ns}	11.84 ± 0.755^{ns}	6.86 ± 0.309^{ns}	1.43 ± 0.044^{ns}	14.34 ± 0.44^{ns}
Group III - (VPA 400 mg/kg)	9.287 ± 0.905$^{###}$	27.24 ± 1.508$^{###}$	4.02 ± 0.239$^{###}$	0.56 ± .037$^{###}$	7.5 ± 0.445$^{###}$
Group IV - Low Dose (VPA+Resv 10 mg/kg)	14.8 ± 0.814*	18.13 ± 1.403***	6.37 ± 0.371**	1.03 ± 0.08***	10.44 ± 0.592**
Group V – High Dose (VPA+Resv 40 mg/kg)	21.65 ±0.752***	14.26 ± 0.823***	8.06 ± .606***	1.32 ± .026***	11.86 ± 0.547***

Values are expressed as mean ± SEM of n=6 animals. Superscript letters represents the statistical significance done by ANOVA, followed by Tukey's tests. ###P<0.001 Compared with Vehicle; *P<0.05, **P<0.01, ***P<0.001 Compared with Negative Control, ^{ns}=Not significant Compated with Vehicle. Total protein: F(4,10)=51.18, AchE: F(4,17)=38.95, Glutathion: F(4,20)=18.92, Catalase: F(4,20)=54.11, SOD: F(4,19)=33.19.

RESULTS

Behavioral studies

Effect of resveratrol on negative geotaxis in VPA treated mice

There was a significant increase in time taken to re-orient along the inclined plane was observed in Negative control (N.C) group on PND 14 to 19 compared with the control (P<0.001). Resveratrol treated groups (10 mg/kg, 40 mg/kg) showed in decreased time to re-orient when compared with negative control group (P<0.01) during the same period. The reorientation time was consistent from PND 18 (Fig. 1).

Effect of resveratrol on motor activity in the rotarod test

In NC group showed a shorter time to fall from the rotating rod than control group (P<0.001). Treatment with low dose Resveratrol (10 mg/kg) and high dose (40 mg/kg) groups exhibited a longer retention on the rotating rod (P<0.01, P<0.001) compared to VPA treated group on PND 24, 25 and 26 (Fig. 2).

Effect of resveratrol on locomotor activity using actophotometer

Actophotometer is used to evaluate locomotor activity in mice. In NC group, there was increased locomotion due to hyperactive nature compared with control group (P<0.001). VPA induced change in locomotion did not alter much in Low dose (10 mg/kg) (P<0.05, P<0.01), however it did alter significantly in high dose Resveratrol group (40 mg/kg) with decreased hyperactivity (P<0.001) compared with NC group on PND 34, 35, 36 and 37 (Fig. 3).

Effect of resveratrol on thermal nociception in VPA treated mice

There was a significant increase in latency to withdraw the paw in N.C group compared with the control (P<0.001) on PND 37, 38 and 39. In low dose of Resveratrol (10 mg/kg) treated mice there was no significant alteration in paw withdrawal compared to NC group (P<0.05, P<0.01). In high dose (40 mg/kg) there was significant decrease in latency to withdraw the paw (P<0.001) (Fig. 4).

Effect of resveratrol on anxiety using elevated plus maze test in VPA treated mice

In NC group alone spent less time in the open arms of elevated plus maze, compared with control group with P<0.001. Following the treatment with Resveratrol low dose (10 mg/kg) showed moderate significance (P<0.05, P<0.01) and high dose (40 mg/kg) animals elicited a significant increase in the number open arm entries and time spent in the open arms compared with NC group (P<0.001) (Table 1).

Effect of resveratrol on exploratory behavior in the open field activity in VPA treated mice

Open field test used to evaluate patterns of exploration. Exploration exhibited by NC group was significantly lower compared to the control (P<0.001), measured using line crossings and head dipping's. Low dose Resveratrol (10 mg/kg) treatment showed moderate significance (P<0.01) and high dose (40 mg/kg) treated animals showed improvement than NC group (P<0.001) in exploratory behavior as evident in increase in head dipping's in a square open field on PND 40 shown in Fig. 5.

Effect of resveratrol on learning and memory in morris water maze

Mice were allowed to navigate the maze and locate the submerged platform within three trials on each of the three consecutive days PND 38, 39 and 40. There was a significant increase in latency to find the platform in the NC group compared with the control (P<0.001) on PND 37, 38 and 39. In low dose of Resveratrol (10 mg/kg) and high dose (40 mg/kg) treated mice required less time in finding the platform compared to NC group (P<0.05, P<0.01, P<0.001) as shown in Fig. 6.

Biochemical estimations

Effect of resveratrol on total protein

Total protein levels were significantly decreased in NC group when compared with the vehicle treated control group with P<0.001. Although there was no significant increase in Total protein in low dose (10 mg/kg) group (P<0.05), high dose Resveratrol showed a significant increase when compared against NC group (P<0.001) (Table 2).

Effect of Resveratrol on AchE in VPA treated mice

Administration of VPA significantly (P<0.001) increased the acetyl cholinesterase activity in NC group compared with the control group. Resveratrol treatment significantly attenuated the increase in enzyme level induced by VPA with P<0.001 in both Resveratrol 10 mg/kg and 40 mg/kg treated group on comparing with negative control group (Table 2).

Effect of resveratrol on glutathione

In NC group have exhibited decreased glutathione activity when compared with the vehicle treated control group with P<0.001. Resveratrol treated animals with 10 mg/kg showed improved glutathione level with P<0.01 and high dose of Resveratrol treated group showed a significant increase of P<0.001 when compared against NC group (Table 2).

Effect of resveratrol on catalase

Administration of VPA significantly decreased the catalase activity when compared with the vehicle treated control group with P<0.001. There was significant increase in catalase enzyme activity in low dose (10 mg/kg) and high dose (40 mg/kg) group when compared against NC group (P<0.001) (Table 2).

Effect of resveratrol on superoxide dismutase

Administration of Valproic acid in negative control group significantly (P<0.001) decreased the superoxide dismutase activity when compared with the control group. Treatment with Resveratrol low and high doses showed an increase in activity of superoxide dismutase compared to NC group significantly with P<0.01 and P<0.001 respectively. Table 12 shows the effect of Resveratrol on superoxide dismutase activity in different treatment groups of animals (Table 2).

Effect of resveratrol on cerebellar purkinje cells

Administration of Valproic acid to the negative control group decreased the Purkinje cell number when compared with the control group. Treatment with Resveratrol low and high doses showed an increase in purkinje cells number when compared to negative control group.

Histopathology

Purkinje cells are GABAergic neurons, releases GABA which exerts inhibitory actions thereby reduces the transmission of nerve impulses. These inhibitory functions enable Purkinje cells to regulate and coordinate motor movements. Purkinje cells are crucial in cerebellar function. The loss of Purkinje cells has

been observed in children with autism. The cerebellar cortex is made up of three layers, consisting of an outer synaptic layer (also called the molecular layer), an intermediate discharge layer (the Purkinje layer), and an inner receptive layer (the granular layer).

Histopathological examinations are carried out by sagittal sections of cerebellum, Control group (H&E ×200) (a), Valproic acid group (H&E × 200) (b) and Resveratrol treated group (H&E×200) (c and d) showing distribution of Purkinje cells. Investigations of cerebellum revealed that Purkinje cells layer in the control group (Fig. 7a) are healthy and normal. A diminished number of Purkinje cells layer seen in negative control group (Fig. 7b). Low dose and high doses of resveratrol treatment (10 & 40 mg/kg) restored the Purkinje cell layer, granular layer and molecular layer damage in a dose dependent manner (Fig. 7c and d).

DISCUSSION

Thus the results of our study demonstrated that, valproic acid significantly developed the autistic features in mice, behavioral findings are done on PND 14 to 40. Negative geotaxis is used to assess sensory motor functions of infant mice across an inclined plane. It is the movement of mice in response to the gravity stimulus and is considered as a diagnostic feature of vestibular function, a measure of sensory motor function. Valproic acid group animals showed an increased latency time to re-orient themselves when they placed along an inclined plane could be due to vestibular damage. [18] Administration of Resveratrol significantly reduced the reorientation time showed its protective effect towards vestibular damage.

Cerebellum plays an important role in motor skills development. Decreased motor performance, which is a noticeable symptom in autism due damage to cerebellar region. This is tested by using rotarod. Valproic acid alone group animals were showed decreased retention time on rotating rod. [19] Treatment with resveratrol improved the muscular coordination and increased retention time on rotating rod in comparison with valproic acid; considering this resveratrol having the protecting activity on cerebellum.

Hyperactivity is a significant feature of autism. Exposure of Valproic acid causes hyperactivity in mice, and they are actively crossing the photo beams exhibiting increased locomotion in a novel environment. Understanding anxiety in elevated plus maze is similar to that seen in autistic subjects. [32-33] Anxiety is due to increased glutaminergic transmission in brain. Valproic acid impairs the functionality and morphology of the glutaminergic neuronal networks associated with hyper excitability, fear and anxiety. Treatment with resveratrol increased the number of open arm entries and time spent in open arm than closed arm in elevated plus maze implying that decreased fear and anxiety when compared to Valproic acid. Valproic acid disrupts the pain modulating pathways. [34] Hot plate was used to test thermal nociception. One of autistic feature is response towards pain stimuli less. Valproic acid significantly increased the pain threshold. Treatment with resveratrol significantly decreased the threshold for pain perception due to protective effect in pain related pathways and alerting sensory neurons.

In autism there is decreased motivation to explore a new environment is observed. Valproic acid cause's decreased head dipping's and line crossings. Treatment with resveratrol normalized exploratory activity comparison to valproic acid group. Lack of spatial organization skills that is observed in autism, oxidative stress can also lead to memory impairment. [35] Valproic acid increased the latency to swim to a hidden platform in the water maze test. Administration of resveratrol improved the navigation skills & cognition enhancing which might be due to its protecting neuron density in hippocampus.

Acetylcholine is principle neurotransmitter concerned with learning and memory process, and it is hydrolyzed by an enzyme acetyl cholinesterase (AchE). [36] Valproic acid causes oxidative stress that lead to up regulation of acetyl cholinesterase enzyme in neuron; this reduces the concentration of acetylcholine. More acetylcholine is made available for the receptor that improves cognitive process. Resveratrol significantly decreased the AchE levels when compared valproic acid group is observed.

Valproic acid decreased the antioxidant enzymes SOD, Catalase, glutathione, and increased lipid peroxidation process. This cytotoxic activity of valproic acid is due to the generation of hydrogen peroxide and hydroxyl free radicals. [37] Resveratrol significantly decreased these reactive oxygen species (ROS) and proved it potent antioxidant property towards oxidative stress. [38]

One of the reliable biological findings of autism is the significant reduction (35–50%) in the number of purkinje cells within the cerebellum of brain. Histopathological studies showed that valproic acid administration damaged the Purkinje cell layer and granule cell death in cerebellum. Purkinje cell layer integrity was restored with resveratrol treatment indicating to its neuroprotective action. [39]

On the basis of these findings, we concluded that Resveratrol is a potent antioxidant, cognition enhancing, and neuroprotective molecule in valproic acid induced oxidative stress markers, behavioural deficits, altered brain neurotransmitters, and altered Purkinje cell distribution. Resveratrol pharmaceutically useful as antioxidant against environmental stresses, protect nerve cells from damage.

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