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**Medroxyprogesterone acetate
improves propionic acid-induced
autism in rat model and magnetic
resonance spectroscopic correlation**

Medroxyprogesterone acetate improves propionic acid-induced autism rat model and magnetic resonance spectroscopic correlation

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Abstract

The protective effect of medroxyprogesterone acetate on propionic acid-induced autism in rats was evaluated. For this purpose, 30 rats were divided into three groups. The significant difference in the levels of IL-17 ($p < 0.05$), IL-2 ($p < 0.05$), and TNF- α ($p < 0.05$), lactate ($p < 0.05$), and nerve growth factor ($p < 0.05$) were found in the medroxyprogesterone-treated group by biochemical analysis. In histopathological examination, the medroxyprogesterone-treated group revealed significant improvement in neural body degeneration, neural count, and dysmorphological changes in both CA1 and CA3 regions. Immunohistochemical examination revealed improvement in glial activity with glial fibrillar acidic protein and morphological changes in Purkinje cells. Magnetic resonance spectroscopy showed an improvement in the level of lactate duplets in the medroxyprogesterone-treated group. To our knowledge, this is the first study, to evaluate the protective effect of medroxyprogesterone on propionic acid-induced autism in rats.

Introduction

Autism spectrum disorder is a disorder of the brain system caused by the abnormal development of the brain. There are seven commonly used classes of medication for its treatment. These are tranquilizers/antipsychotics (risperidone, olanzapine, quetiapine, and ziprasidone), antidepressants (paroxetine, sertraline, fluoxetine, and citalopram hydrobromide), stimulants (methylphenidate, dextroamphetamine, dexamethylphenidate, and modafinil), anxiolytic/sedative/hypnotics, hypotensive agents (buspirone, hydroxyzine, and zolpidem), benzodiazepines (benzodiazepines were lorazepam, diazepam, and alprazolam) and anticonvulsants (divalproex sodium, topiramate, lamotrigine, and gaba-

pentin) (Oswald and Sonenklar, 2007). Although these drugs have a positive role against autism spectrum disorder but also have some negative effects on different organs. Some natural neuroprotective agents should be searched for the treatment of these diseases.

Medroxyprogesterone acetate is a synthetic progesterone and is used mostly as a contraceptive and hormone replacement. It interacts with the glucocorticoid receptors (Gerhard et al., 1998) and also has anti-inflammatory effects (Wakatsuki et al., 2002). Endometriosis was successfully treated with medroxyprogesterone (Haney and Weinberg, 1988). Medroxyprogesterone is involved in the down-regulation of Th1, Th17 and up-regulation of Th22 which are involved in the process of infection



and inflammation (Piccinni et al., 2019). It showed an agonist effect in estrogen treatment in macaques (Pazol et al., 2004). Its antagonistic effect on androgen receptors has been reported in studies on humans (Bentel et al., 1999). Due to these anti-inflammatory properties and the strong effect of medroxyprogesterone on androgen receptors and different hormones, we hypothesized that medroxyprogesterone can improve propionic acid-induced autism model in rats. The magnetic resonance spectroscopy study of the brain was also conducted.

Materials and Methods

In this experimental study, 30 male albino rats were included weighing 200-250 g at 12-14 weeks old. The experimental Animal Lab of this University provided these rats. Rats were housed in special steel cages and *ad libitum* food was provided during the experiment. A controlled environment was provided to the rats with a temperature of $24 \pm 2^\circ\text{C}$ and light with 12-hour period cycles.

Propionic acid was given intraperitoneally 250 mg/kg/day to rats (n=20) over 5 days to induce an autism model. Ten rats were used as a control group. The rats given propionic acid were then randomly divided into 2 groups.

There were in total 3 groups, control (Group I), propionic acid (Group II), and propionic acid plus medroxyprogesterone (Group III). Group 1 (n=10) received only saline. Group 2 (n=10) received propionic acid (via oral gavage 1 mL/kg/day) and saline. Group 3 (n=10) received propionic acid (via oral gavage 1 mL/kg/day) and medroxyprogesterone (via oral gavage 2 mg/kg/day, Tarlusal tablet 5 mg, Deva). The experiment was completed in 15 days. At the end of 15 days, behavioral tests were done. Behavioral tests were performed between 10:00 AM and 3:00 PM. Finally, rats were dissected under general anesthesia. Brain and blood samples were collected for histopathological and biochemical processes.

Behavioral experiments

Box 1: Open field test

Principle

This test was performed to evaluate different behavior, repeated self-grooming, and reduction in search activities in autistic rats.

Requirement

Open air box (50 cm x 50 cm x 40 cm)

Procedures

Step 1: After putting inside the center of the box, the explorational activity of rats was recorded for 5 min manually

Three-chamber sociability and social novelty test

This test was done according to the description mentioned elsewhere (Erbas et al., 2018). Special plexiglas cage was used and the diameter of the cage was 40 cm x 90 cm x 40 cm. The first session was the pre-test session and was performed for 5 min on day 1. After 24 hours of testing sociability, the stranger rat was used and placed inside the cage. In the first session test, the rat was used and placed in the central chamber of the cage. The first session of 10 min was completed after the recording of time spent in each region by the test rat. For the removal of olfactory stimuli of the used rat, the floor of the chamber was washed and cleaned properly before each experiment. The percentage of time spent with the stranger rat was considered significant.

Passive avoidance learning test

A passive avoidance learning test was performed according to the guidance described elsewhere (Erbas et al., 2014). This test is used for the evaluation of the learning and memory abilities of the rat. A box with the dimension of 20 cm x 20 cm x 20 cm was used. The box had dark and bright compartments. The rats were placed in the bright chamber and they like to go toward the dark compartment. Both the dark and light compartments were separated by a door. After crossing that door from the bright to dark compartment, the door was closed and 1.5 mA electric shock was given to the rat for 3-5 sec. The rat was taken from the dark compartment and placed in the cage. After 24 hours, the rat was placed again in the bright compartment and the time for crossing the bright compartment towards the dark compartment was recorded. This time is also called the latency period and this period was recorded for 300 sec. This time was considered as an index of the memory of rats.

Biochemical analysis of tissues

Brain tissues and blood samples were taken from the rats after dissection. Brain tissues were homogenized and centrifuged for 15 min for tissue analysis. Total protein concentrations were evaluated from the brain homogenized solution. All this process was done according to the method described elsewhere using serum albumin (Bradford, 1976). TNF- α , nerve growth factor

without using any software.

Step 2: For the removal of olfactory stimuli of used rat, the floor of the chamber was washed and cleaned properly before each experiment.

Calculation

The number of floor division crossed with paws is called ambulation and the total ambulation were considered significant.

References

Erbas et al., 2018

(NGF), IL-17, and IL-2 and lactate levels of brain tissues were evaluated with the help of enzyme-linked immunosorbent assay (ELISA) kits. All the process of ELISA was done according to the guidelines of the manufacturer Company. For the measurement of absorbance, a microplate reader (MultiscanGo, Thermo Fisher Scientific Laboratory Equipment, USA) was used.

Hippocampus and cerebellum histopathology

The cornu ammonis (CA) 1 and CA 3 regions of hippocampus and cerebellum were chosen as the target areas to be examined for hippocampus damage. Following behavioral tests, animals were euthanized and their brains removed and fixed for 3 days in a 10% neutral buffered formaldehyde solution. Then, they were moved into 30% sucrose and stored at 4°C until infiltration was complete. The brains were cut coronally on a sliding microtome at 4 µm and mounted on silanized glass slides. For glial fibrillar acidic protein immunohistochemistry, brain sections were incubated with H₂O₂ (3%) for 20 min to eliminate endogenous peroxidase activity, and blocked with serum blocking solution (Ultra V Block, TA-060-UB, Thermo-Scientific) for 20 min at room temperature. Subsequently, sections were incubated in primary antibodies against glial fibrillar acidic protein (1/50 Thermo Scientific, RB-087) for 24 hours at 4°C. Antibody detection was performed with the ABC kit (Vectastain Elite ABC-HRP Kit, PK-6101, Vector Laboratories) and 3-amino-9-ethylcarbazole (AEC, TA-125-HA Thermo-Scientific) was used to visualize the final product. All slides were photographed with an Axiocam ICc 5 digital camera mounted on Zeiss Lab.A1 microscope and images were analyzed with ZEN2 image analysis system and image J. All histopathological examinations were performed by the same investigator who was blinded to the study groups.

Cerebellum measurements: Purkinje cells with clearly distinguishable nuclei in 10 cerebellar lobules were counted and averaged in 4 random H&E stained sections. At the same time, the longest diameter of the nucleus of 100 Purkinje cells was measured. Glial fibrillar acidic protein positive cells per unit area were counted in 5 different lobules in the grisea layer of the cerebellum in glial fibrillar acidic protein stained slides (Arafat and Shabaan, 2019; Celik et al., 2018; El-Eraky El-Azab et al., 2018).

Hippocampus measurements: in 4 random sections, neurons with distinguishable nuclei and glial fibrillar acidic protein positive astrocytes were counted in CA1 and CA3 regions of the hippocampus.

Magnetic resonance spectroscopy of brain

An automated multivoxel 2D chemical shift imaging sequence (TR = 1000 ms; TE = 35 ms; phase encoding $x = 24$; phase encoding $y = 24$; number of excitation pulses = one) was used for ¹H-MRS. FOV (60 mm), slice thickness (4 mm) and the voxel size of the MRS (1.87 × 1.87 × 4 mm³) were used. Tumor and ¹H-MRS were placed

by T₂ weighted imaging, and shimming was done with the help of MRI scanner. The volume of interest was chosen within the right striatum. The result of duration for 2D ¹H-magnetic resonance spectrum acquisition was 580 sec. Magnetom software (Siemens Healthcare) was used to process all the data.

Statistical analysis

SPSS version 15.0 (Chicago, IL, USA) was used to perform statistical analysis of this experimental study. Normality and homogeneity of variances were evaluated by performing Shapiro-Wilk's and Levene's tests. Data results were shown as mean ± standard error of the mean (SEM). P≤0.05 value was considered as significant.

Results

Sociability tests results

The percentage of the time spent with stranger rat was found significantly higher in the control group and propionic acid + medroxyprogesterone group (p<0.05), compared to the propionic acid group. The number of ambulation in open field test was found less in propionic acid group compared to control group and propionic acid + medroxyprogesterone group (p<0.05). The passive avoidance latency was evaluated and there was a significant negative correlation between the propionic acid group and propionic acid + medroxyprogesterone group (p<0.05) (Table I).

Biochemical results

The level of brain TNF-α was found significantly lower in the control group and propionic acid + medroxyprogesterone groups (p<0.05) compared to the propionic acid group in rats. The levels of IL-17 and IL-2 were also high in propionic acid group compared to control group and propionic acid + medroxyprogesterone group (p<0.05). The brain lactate level was measured and there was a significant negative correlation between medroxyprogesterone group (p<0.05) propionic acid group and other groups. The results for the NGF level of brain were evaluated in rats. The level of the brain NGF was significantly low compared to control and propionic acid + medroxyprogesterone groups medroxyprogesterone group (p<0.05) (Table I).

Histopathological, immunohistochemical, and magnetic resonance spectroscopy results

Neuronal counts in CA1 and CA3 regions of hippocampus were evaluated and a reduced number of neurons were found in the propionic acid group compared to the control and propionic acid + medroxyprogesterone groups (p<0.05). Purkinje cell count in the cerebellum was done and this count was low in propionic acid group when we compared to the control and propionic acid + medroxyprogesterone groups medroxyprogesterone group (p<0.05). The results of immunostaining

Table I
Effects of medroxyprogesterone on different parameters in rat brain

Parameters	Control	Propionic acid	Propionic acid + medroxyprogesterone
Sociability test (%time spent with the stranger rat)	72.3 ± 8.4	36.6 ± 5.9 ^a	61.5 ± 4.6 ^b
Open field test (total ambulation)	20.7 ± 2.9	6.7 ± 1.8 ^a	17.1 ± 3.9 ^a
Passive avoidance learning test (latency period in sec)	244.8 ± 28.9	106.4 ± 33.5 ^b	113.5 ± 24.7
<i>Biochemical analysis of brain tissue</i>			
TNF-alfa (pg/mg protein)	18.2 ± 4.1	78.8 ± 6.0 ^a	49.5 ± 7.8 ^c
IL-2 (pg/g protein)	2.2 ± 0.4	318.7 ± 11.5 ^b	98.6 ± 3.7 ^d
IL-17 (pg/g protein)	233.2 ± 18.7	523.4 ± 30.3 ^b	379.8 ± 19.5 ^d
Lactate (mmol/100 g wet weight)	1.3 ± 0.1	3.24 ± 0.3 ^a	1.64 ± 0.2 ^c
NGF (pg/mg protein)	75.5 ± 8.1	33.2 ± 3.8 ^b	54.4 ± 9.9 ^c
<i>Histopathological and immunohistochemical data</i>			
Neuronal count CA1	81.9 ± 6.7	54.5 ± 5.7 ^b	69.4 ± 7.5 ^c
Neuronal count CA3	43.8 ± 5.9	31.2 ± 6.6 ^a	38.9 ± 0.9 ^c
GFAP immunostaining index (CA1)	30.8 ± 1.4	66.8 ± 8.5 ^b	46.1 ± 4.4 ^c
GFAP immunostaining index (CA3)	35.1 ± 5.5	65.4 ± 4.2 ^a	48.8 ± 7.5 ^c
Purkinje count cerebellum	21.3 ± 2.4	11.5 ± 2.9 ^a	17.7 ± 0.7 ^d
GFAP immunostaining index (cerebellum)	42.2 ± 6.6	55.1 ± 2.4 ^a	44.7 ± 3.1 ^c
Magnetic resonance spectroscopy lactate value (%control)	100	345.8 ± 67.7 ^b	131.4 ± 15.5 ^d
Data results were showed as mean ± SEM. One-way ANOVA was used. ^a p<0.01, ^b p<0.001 significant difference from normal groups; ^c p<0.05, ^d p<0.001 significant difference from propionic acid and saline group			

of glial fibrillar acidic protein were evaluated in CA1 and CA3 regions of the hippocampus and cerebellum region of the brain. The immunopositivity of glial fibrillar acidic protein was significantly higher in the propionic acid group compared to the control and propionic acid + medroxyprogesterone groups medroxyprogesterone group ($p<0.05$) in both cerebellum and hippocampus part of the brain in rats. Lactate value percentage was evaluated with magnetic resonance spectroscopy and the results were significantly higher in the propionic acid group compared to the control and propionic acid + medroxyprogesterone groups in the brain part of rat medroxyprogesterone group ($p<0.05$; Table I).

The neuronal counts in CA1 and CA3 regions of the hippocampus were evaluated using a cresyl violet stain. Normal pyramidal neurons were found in the control group. The propionic acid group showed neural body degeneration and decreased neural count and dysmorphological changes in both CA1 and CA3 regions. Propionic acid + medroxyprogesterone group revealed increased neural count and improved neural morphology changes in CA1 and CA3 regions (Figure 1).

Glial fibrillar acidic protein immunostaining in the CA1 and CA3 regions of the hippocampus was evaluated. The glial activity in the propionic acid group was found higher compared to the control and propionic acid + medroxyprogesterone groups in both the CA1 and CA3 regions of the hippocampus in rats (Figure 2).

The cerebellum was stained with hematoxylin and eosin stain and glial fibrillar acidic protein immunostaining. The control group revealed normal morphology of Purkinje neurons. The propionic acid group revealed increased glial activity with glial fibrillar acidic protein and dysmorphological changes in Purkinje neurons. Propionic acid + medroxyprogesterone group revealed decreased glial activity with glial fibrillar acidic protein and improved Purkinje neural morphology in the cerebellum of rats (Figure 3).

Lactate duplets were evaluated with magnetic resonance spectroscopy and the results were significantly higher in the propionic acid group compared to the control and propionic acid + medroxyprogesterone groups in the brain part of rats (Figure 4).

Discussion

This study demonstrated that medroxyprogesterone has a positive role in the improvement of propionic acid-induced autism model in rats due to its anti-inflammatory effect and strong association with androgen receptors and hormones. Propionic acid-induced autistic rats revealed abnormal sociability tests. Significant changes in the behaviors of rats were monitored in the medroxyprogesterone-treated group. This group revealed higher number of normal neuronal cells as compared to the propionic acid group and also improvement in the morphology of these cells in both

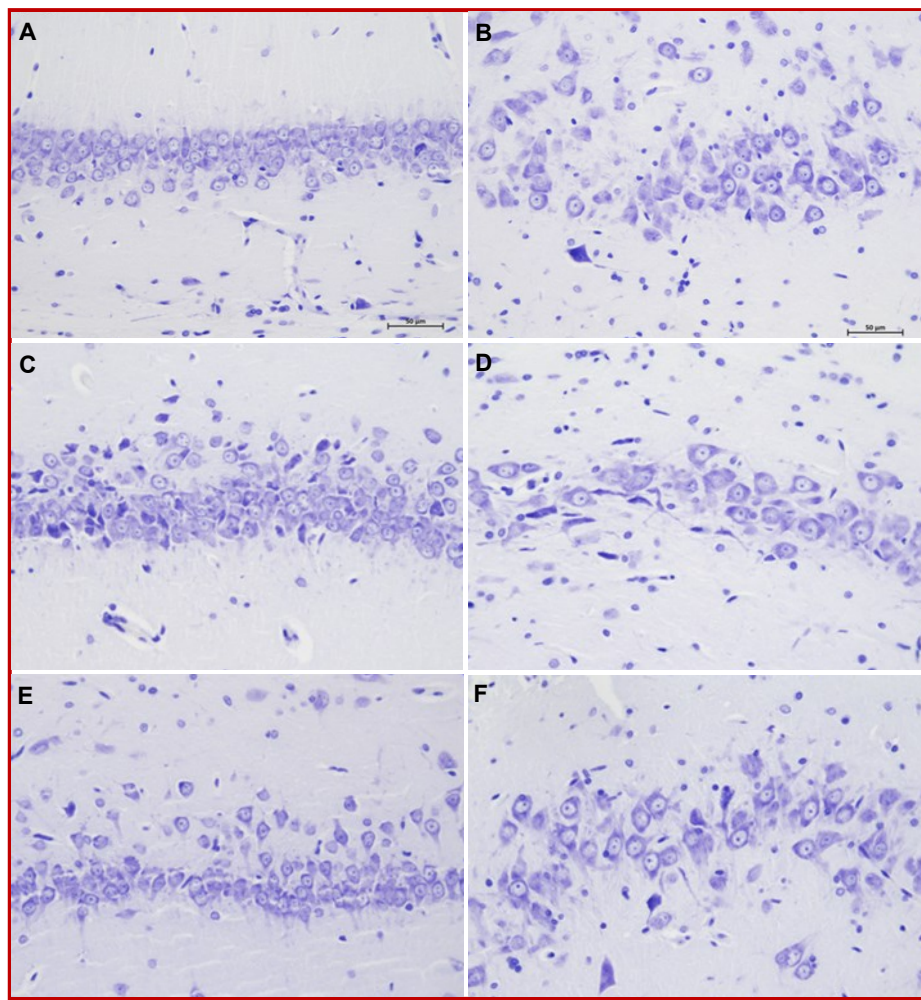


Figure 1: Microscopic view of cresyl violet stained hippocampus. Normal control group male rats CA1 and CA3. Normal pyramidal neuron (A-B); propionic acid and saline group male rats have neural body degeneration and decreased neural count and dysmorphological changes in CA1 and CA3 (C-D). propionic acid and medroxyprogesterone group male rats have increased neural count and improved neural morphology changes in CA1 and CA3 (scale bars = 50 μm) (E-F)

CA1 and CA3 regions was evaluated. Decrease in the astroglial activity in the CA1 and CA3 region and improvement in the morphology of Purkinje cells were also evaluated in rats of the medroxyprogesterone group. Improved results of lactate duplets were found in the medroxyprogesterone group compared to the propionic acid group.

Autism spectrum disorder is a brain disorder associated with neuroinflammatory changes in different parts of the brain. Different genetical models have been studied to understand the possible causes of this disease (Bill and Daniel, 2009). Endocrine disrupting compounds may affect the development of autism by disrupting the *in utero* hormonal milieu directly or changing the metabolism and action of maternal hormones (Braun, 2012). In another study, it was proposed that autism may give a unique insight into the genetic and developmental processes of the brain. It can shape early neural wiring patterns and make possible the process of

socialism and communication (Courchesne et al., 2007).

Activation of astrocytes and microglial cells, higher levels of inflammatory cytokines, and other factors are indicators of neuroinflammation in autistic patients (Kern et al., 2016). Higher levels of activated microglia and astrocytes in the hippocampus, white matter, and the neocortex have been reported in the autopsy of an autistic patient (Bauman and Kemper, 2005; Vargas et al., 2005). Both young and old patients showed these brain findings. It has been suggested that neuroinflammation may be present throughout the life of an autistic patient. The high reactivity of the immune system has been evaluated in the monocytes of peripheral blood of autistic patients (Molloy et al., 2006). Evidence for peripheral immune activity was given because it may relate to the increased activity of microglia in the brain of autistic patients. These patients may present the peripheral macrophages after the migration into the brain. These cell types increase

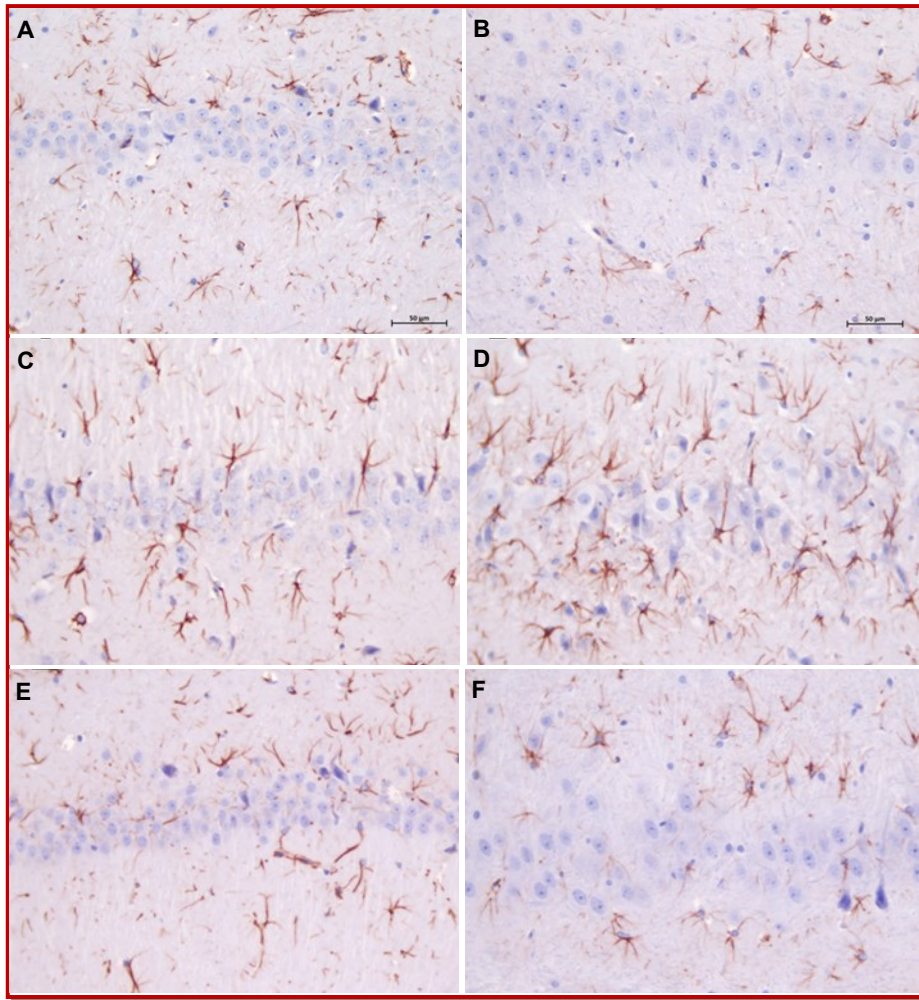


Figure 2: Microscopic view of glial fibrillar acidic protein stained CA1 and CA3 regions of hippocampus. Astrogliosis was characterized by glial fibrillar acidic protein immunostaining. Normal control group male rats CA1 and CA3 (A-B); propionic acid and saline group male rats have increased astroglial activity CA1 and CA3 (C-D); propionic acid and medroxyprogesterone group male rats have decreased astroglial activity CA1 and CA3 (scale bars = 50 μ m) (E-F)

inflammatory cytokines, reactive oxygen species (i.e nitric oxide and hydrogen peroxide), and oxidative stress in the brain tissues (Dringen, 2005). Similar effects (Le Poul et al., 2003) and higher levels of inflammatory cytokines, tumor necrotic factors, and chemoattractant protein of macrophages have been reported in autism spectrum disorder (Vargas et al., 2005). A positive correlation between autism-induced behaviors and serum level of inflammatory cytokines in 22q11.2 deletion syndrome has been reported in a previous study (Ross et al., 2013).

The biochemical results of this study are similar to previous studies of autism spectrum disorder in which we found the higher level of IL-17, IL-2, TNF- α , lactate, and NGF in the propionic acid group. This indicated neuroinflammation in the brain. The protective and anti-inflammatory role of medroxyprogesterone have been reported in demyelinating disorders in a mouse model. A decrease in the levels of microglial markers (IL- β , TNF- α , iNOS) and demyelination was evaluated in that

study (Mohammadi et al., 2021). The anti-inflammatory properties of medroxyprogesterone have been reported in many studies (Mohammadi et al., 2021; Wakatsuki et al., 2002; Haney and Weinberg, 1988; Piccinni et al., 2019) and based on these properties it was hypothesized that medroxyprogesterone acetate may be used to protect the propionic acid-induced autism model in rats. N-acetylcysteine has also been reported to protect the brain against propionic acid-induced neurotoxicity in rats due to its antioxidant and anti-inflammatory properties (Al-Dbass, 2014).

Behavioral disability, including abnormal activities during playing and many other abnormal forms of social activities, are the main findings of autism spectrum disorder (Shultz et al., 2008). Many studies have been reported to evaluate the results of propionic acid on the social behavior of rats (Shultz et al., 2008; MacFabe et al., 2011). Propionic acid-treated rats revealed abnormal social behavior that was reported by the longer mean distance apart, less time spent in a close

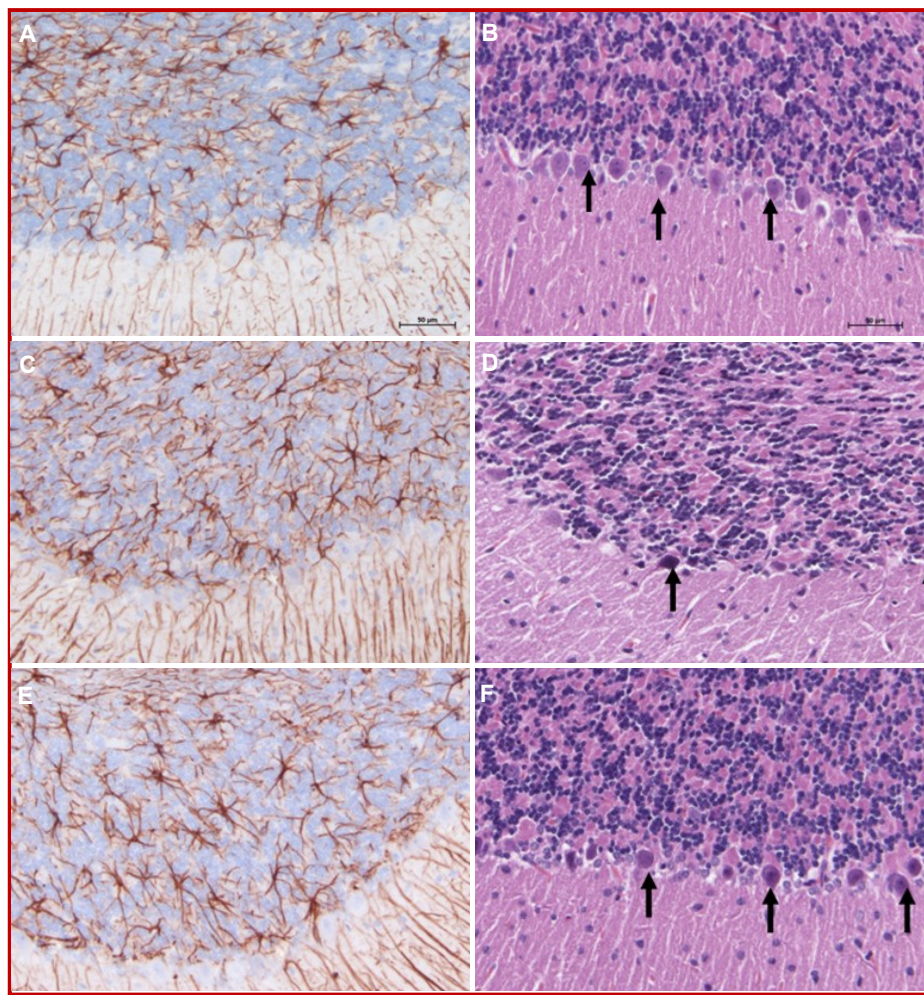


Figure 3: Microscopic view of glial fibrillar acidic protein (A-C-E) and H&E stained (B-D-F) cerebellum. Astrogliosis was characterized by glial fibrillar acidic protein immunostaining (Red-Brown staining). Normal control group male rat's cerebellum and normal Purkinje cells (arrows) (A-B); propionic acid and saline group male rats have increased astroglial activity in the cerebellum, and degenerative changes in Purkinje cells (arrows) (C-D); propionic acid and medroxyprogesterone group male rats have decreased astroglial activity in the cerebellum, and improved Purkinje cell morphology (arrows) in cerebellum (scale bars = 50 μm) (E-F)

area, less interaction and abnormal playing actions (Shultz et al., 2008). Sodium acetate is another fatty acid that also has a negative impact on the social behavior in rats (MacFabe et al., 2011). The abnormalities in the social behavior of rats were similar in both autism spectrum disorder and the propionic acid-treated rats (Shultz et al., 2008). Social behavioral tests have been tested in other studies (Moy et al., 2004; Silverman et al., 2010). The rats with autism spectrum disorder revealed an abnormal approach to an unknown rat and preference for social novelty was also reduced (Bambini-Junior et al., 2011; MacFabe et al., 2011).

Open field assay has also been reported in different rat models of autism spectrum disorder and hyperactivity was evaluated in the autistic rats (Narita et al., 2010; Schneider and Przewlocki, 2005). The behavioral tests have also been reported in different studies of rats

(Erbaş et al., 2018; Erbaş et al., 2014).

Propionic acid and other fatty acids relevant to the process of autism spectrum disorder have been reported to induce neuroinflammatory processes (MacFabe et al., 2007; Shultz et al., 2008). Neuroinflammation including activated microglia and astrocytes was evaluated in the hippocampus. This neuroinflammatory process can cause abnormal behavior in the propionic acid-induced autism model (Whitton, 2007). Propionic acid-related neuroinflammation was found in the neocortex and hippocampus (Shultz et al., 2008). Rats treated with propionic acid revealed increased activation of microglia and astrocytes. Increased immunostaining of glial fibrillar acidic protein was also evaluated in the brains of propionic acid-treated rats (MacFabe et al., 2007). In a study of valproic acid-induced autism in mice, an increased level of inflammatory cytokines, higher acti-

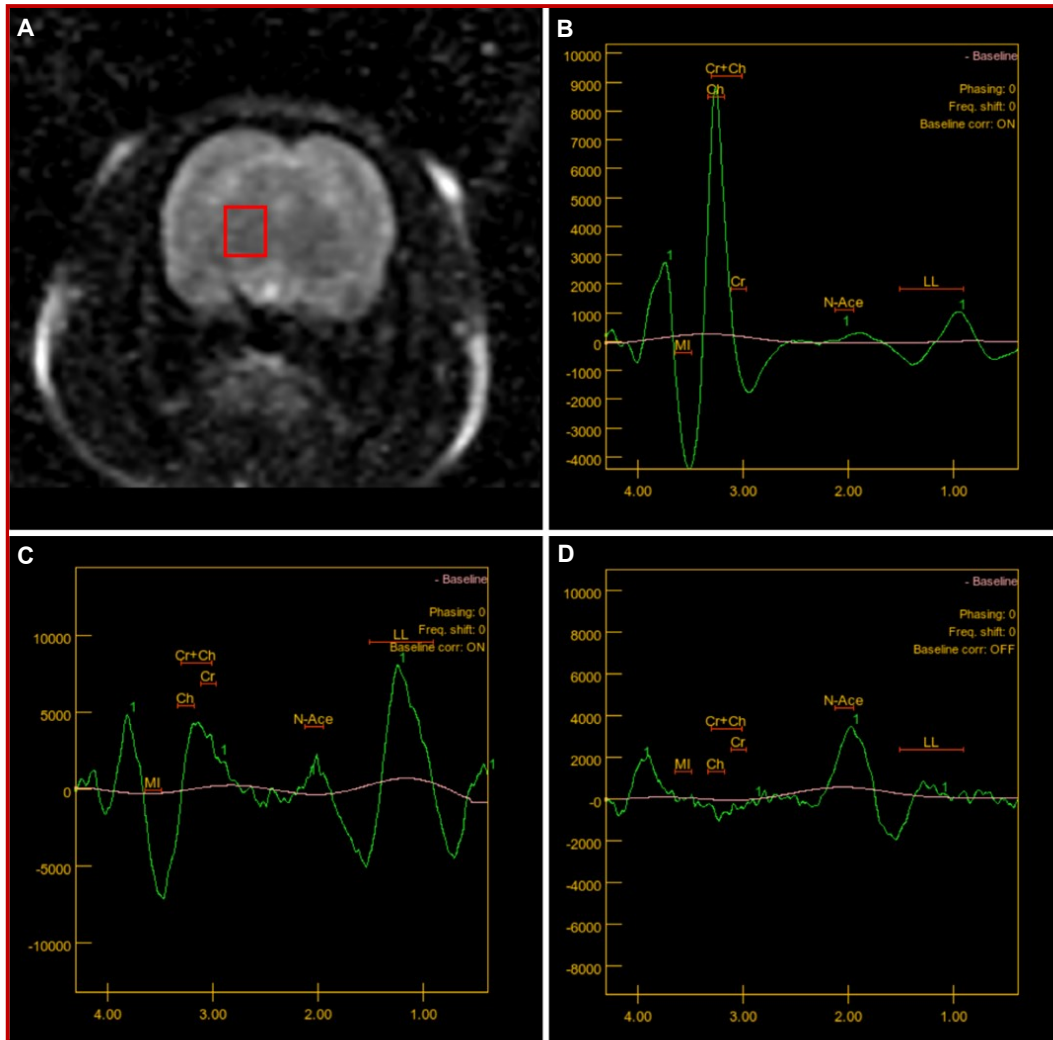


Figure 4: MR spectroscopy. A: MR spectroscopy chosen area (Red box), B: Normal Control Group male Rats, C: PA and saline group male rats, D: PA and MA group male rats

vity of glial cells, and morphological abnormalities in Purkinje cells were described (Al-Gholam and Ameen, 2020). Abnormal behavioral activities, oxidative stress, and abnormalities in Purkinje cells have been reported in another study of autism in mice (Bakshi et al., 2018). Higher activity of inflammatory cytokines (TNF- α , interleukins), abnormal social behavior, higher level of apoptotic markers, and lower level of anti-apoptotic markers and morphological abnormalities were found in the propionic acid-induced autism model of rats (Tiwari et al., 2021). Abnormal social behavior, higher level of anxiety and stress, late response to painful stimulation, and a higher level of oxidative stress markers was evaluated in the study of autistic mice (Al-Amin et al., 2015). Decrease in the number of Purkinje cells, a higher percentage of glial fibrillar acidic protein immunopositivity and a higher level of oxidative stress markers have been found in the autism study of rats (Arafat and Shabaan, 2019). Memory defects, abnormal behavior, neuroinflammatory and neurotransmitter

cells imbalance, oxidative stress, higher level of inflammatory cytokines and brain abnormalities were described in the study of propionic acid-induced autism model of rats (Sharma et al., 2019). All these behavioral, biochemical and histopathological findings related to the autism model are similar to the findings of the current study. Many neuroprotective agents have been reported in the autism model of rats and mice. These neuroprotective agents improved the social behavior, decreased the level of inflammatory cytokines, improved the morphological abnormalities of Purkinje cells, and decreased the percentage of immunopositivity of glial fibrillar acidic protein in hippocampus and cerebellum of autistic rats and mice (Al-Gholam and Ameen, 2020; Bakshi et al., 2018; Tiwari et al., 2021; Al-Amin et al., 2015; Arafat and Shabaan, 2019; Sharma et al., 2019). The improved results of medroxyprogesterone in the cerebellum and hippocampus of rats are quite similar to the results of these studies of neuroprotective agents.

Abnormalities in mitochondrial functioning have been reported to induce autism spectrum disorder in the study of humans. It has been suggested that mitochondrial abnormality may be the neurobiological subtype of autism spectrum disorder (Goh et al., 2014). Abnormal mitochondrial activities have been reported in different studies of autism spectrum disorder (Giulivi et al., 2010; Oliveira et al., 2005; Correia et al., 2006; Tang et al., 2013). Magnetic resonance spectroscopy was evaluated and a higher level of lactate doublets have been reported in the brain part of autism spectrum disorder patient (Goh et al., 2014). The results of magnetic resonance spectroscopy in the current study are similar to these results. Low levels of lactate doublets in magnetic resonance spectroscopy were observed in the brain of the medroxyprogesterone-treated group.

Conclusion

Behavioral, biochemical, histopathological and magnetic resonance spectroscopy results of this study support the positive/improved effect of medroxyprogesterone on the propionic acid-induced autism model in rats.

Financial Support

Self-funded

Ethical Issue

Experimental procedure was followed according to the guidelines of American's national Institute for the care and use of rats. Ethical approval was taken from the Science University with the ethical number (21210901)

Conflict of Interest

Authors declare no conflict of interest

References

- Abdelli LS, Samsam A, Naser SA. Propionic acid induces gliosis and neuro-inflammation through modulation of PTEN/AKT pathway in autism spectrum disorder. *Sci Rep.* 2019; 9: 1-12.
- 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.
- Al-Dbass AM. N-Acetylcysteine reduces the neurotoxic effects of propionic acid in rat pups. *J King Saud Univ Sci.* 2014; 26: 254-60.
- Al-Gholam MA, Ameen O. The neuroprotective effect of Ginkgo Biloba extract on valproic acid induced autistic features in mice. *J Clin Diagnostic Res.* 2020; 1-7.
- Arafat EA, Shabaan DA. The possible neuroprotective role of grape seed extract on the histopathological changes of the cerebellar cortex of rats prenatally exposed to valproic acid: Animal model of autism. *Acta Histochem.* 2019; 121: 841-51.
- Bakshi V, Sunand K, Begum N, Kakalij RM, Tekula MR. Neuroprotective effect of resveratrol on valproic acid induced oxidative stress autism in Swiss albino mice. *Int J Pharm Sci Drug Res.* 2018; 10:103-10.
- Bambini-Junior V, Rodrigues L, Behr GA, Moreira JCF, Riesgo R, Gottfried C. Animal model of autism induced by prenatal exposure to valproate: Behavioral changes and liver parameters. *Brain Res.* 2011; 1408: 8-16.
- Bauman ML, Kemper TL. Neuroanatomic observations of the brain in autism: A review and future directions. *Int J Dev Neurosci.* 2005; 23: 183-87.
- Bentel JM, Birrell SN, Pickering MA, Holds DJ, Horsfall DJ, Tilley WD. Androgen receptor agonist activity of the synthetic progestin, medroxyprogesterone acetate, in human breast cancer cells. *Mol Cell Endocrinol.* 1999; 154: 11-20.
- Bill BR, Geschwind DH. Genetic advances in autism: Heterogeneity and convergence on shared pathways. *Curr Opin Genet Dev.* 2009; 19: 271-78.
- Braun JM. Endocrine disrupting compounds, gonadal hormones, and autism. *Dev Med Child Neurol.* 2012; 54: 1068-68.
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976; 72: 248-54.
- Celik I, Seker M, Salbacak A. Histological and histomorphometric studies on the cerebellar cortex and silver stained nucleolus organizer regions of Purkinje neurons in chronic morphine-treated rats. *Vet Arh.* 2018; 88: 75-88.
- Correia C, Coutinho AM, Diogo L, Grazina M, Marques C, Miguel T, Ataíde A, Almeida J, Borges L, Oliveira C, Oliveira G, Vicente AM. Brief report: High frequency of biochemical markers for mitochondrial dysfunction in autism: No association with the mitochondrial aspartate/glutamate carrier SLC25A12 gene. *J Autism Dev Disord.* 2006; 36:1137-40.
- Courchesne E, Pierce K, Schumann CM, Redcay E, Buckwalter JA, Kennedy DP, Morgan J. Mapping early brain development in autism. *Neuron.* 2007; 224.
- Dringen R. Oxidative and antioxidative potential of brain microglial cells. *Antioxid Redox Signal.* 2005; 74: 374-80.
- El-Eraky El-Azab N, M. El-Mahalaway A, Sabry D. Effect of methyl mercury on the cerebellar cortex of rats and the possible neuroprotective role of mesenchymal stem cells conditioned medium: Histological and immunohistochemical study. *J Gastrointest Cancer Stromal Tumors.* 2018; 8: 2.
- Erbas O, Erdogan MA, Khalilnezhad A, Gürkan FT, Yiğittürk G, Meral A, Taskiran D. Neurobehavioral effects of long-term maternal fructose intake in rat offspring. *Int J Dev Neurosci.* 2018; 69: 68-79.
- Erbas O, Solmaz V, Aksoy D, Yavaşoğlu A, Sağcan M, Taşkıran D. Cholecalciferol (vitamin D₃) improves cognitive dysfunction and reduces inflammation in a rat fatty liver model of metabolic syndrome. *Life Sci.* 2014; 103: 68-72.

- Gerhard M, Walsh BW, Tawakol A, Haley EA, Creager SJ, Seely EW, Ganz P, Creager MA. Estradiol therapy combined with progesterone and endothelium-dependent vasodilation in postmenopausal women. *Circulation* 1998; 98: 11158-63.
- Giulivi C, Zhang YF, Omanska-Klusek A, Ross-Inta C, Wong S, Hertz-Picciotto I, Tassone F, Pessah IN. Mitochondrial dysfunction in autism. *JAMA*. 2010; 304: 2389-96.
- Goh S, Dong Z, Zhang Y, DiMauro S, Peterson BS. Mitochondrial dysfunction as a neurobiological subtype of autism spectrum disorder: Evidence from brain imaging. *JAMA Psychiatry*. 2014; 71: 665-71.
- Haney AF, Weinberg JB. Reduction of the intraperitoneal inflammation associated with endometriosis by treatment with medroxyprogesterone acetate. *Am J Obstet Gynecol*. 1988; 159: 450-54.
- Kern JK, Geier DA, Sykes LK, Geier MR. Relevance of neuroinflammation and encephalitis in autism. *Front Cell Neurosci*. 2016; 9: 519.
- Le Poul E, Loison C, Struyf S, Springael JY, Lannoy V, Decobecq ME, Brezillon S, Dupriez V, Vassart G, Van Damme J, Parmentier M, Detheux M. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *J Biol Chem*. 2003; 278: 25481-89.
- MacFabe DF, Cain DP, Rodriguez-Capote K, Franklin AE, Hoffman JE, Boon F, Taylor AR, Kavaliers M, Ossenkopp KP. Neurobiological effects of intraventricular propionic acid in rats: Possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders. *Behav Brain Res*. 2007; 176: 149-69.
- MacFabe DF, Cain NE, Boon F, Ossenkopp KP, Cain DP. Effects of the enteric bacterial metabolic product propionic acid on object-directed behavior, social behavior, cognition, and neuroinflammation in adolescent rats: Relevance to autism spectrum disorder. *Behav Brain Res*. 2011; 217: 47-54.
- Mohammadi M, Abdi M, Alidadi M, Mohamed W, Zibara K, Ragerdi I. Medroxyprogesterone acetate attenuates demyelination, modulating microglia activation, in a cuprizone neurotoxic demyelinating mouse model. 2021; 10: 57-68.
- Molloy CA, Morrow AL, Meinzen-Derr J, Schleifer K, Dienger K, Manning-Courtney P, Altaye M, Wills-Karp M. Elevated cytokine levels in children with autism spectrum disorder. *J Neuroimmunol*. 2006; 172: 198-205.
- Moy SS, Nadler JJ, Perez A, Barbaro RP, Johns JM, Magnuson TR, Piven J, Crawley JN. Sociability and preference for social novelty in five inbred strains: An approach to assess autistic-like behavior in mice. *Genes Brain Behav*. 2004; 3: 287-302.
- Narita M, Oyabu A, Imura Y, Kamada N, Yokoyama T, Tano K, Uchida A, Narita N. Nonexploratory movement and behavioral alterations in a thalidomide or valproic acid-induced autism model rat. *Neurosci Res*. 2010; 66: 2-6.
- Oliveira G, Diogo L, Grazina M, Garcia P, Ataíde A, Marques C, Miguel T, Borges L, Vicente A.M, Oliveira CR. Mitochondrial dysfunction in autism spectrum disorders: A population-based study. *Dev Med Child Neurol*. 2005; 47:185-89.
- Oswald DP, Sonenklar NA. Medication use among children with autism spectrum disorders. *J Child Adolesc Psychopharmacol*. 2007; 17: 348-55.
- Pazol K, Wilson ME, Wallen K. Medroxyprogesterone acetate antagonizes the effects of estrogen treatment on social and sexual behavior in female macaques. *J Clin Endocrinol Metab*. 2004; 89: 2998-06.
- Piccinni MP, Lombardelli L, Logiodice F, Kullolli O, Maggi E, Barkley MS. Medroxyprogesterone acetate decreases Th1, Th17, and increases Th22 responses via AhR signaling which could affect susceptibility to infections and inflammatory disease. *Front Immunol*. 2019; 10: 1-15.
- Ross HE, Guo Y, Coleman K, Ousley O, Miller AH. Association of IL-12p70 and IL-6: IL-10 ratio with autism-related behaviors in 22q11.2 deletion syndrome: A preliminary report. *Brain Behav Immun*. 2013; 31, 76-81.
- Schneider T, Przewłocki R. Behavioral alterations in rats prenatally to valproic acid: Animal model of autism. *Neuropsychopharmacology*. 2005; 30, 944-57.
- Sharma R, Rahi S, Mehan S. Neuroprotective potential of solanesol in intracerebroventricular propionic acid induced experimental model of autism: Insights from behavioral and biochemical evidence. *Toxicol Reports*. 2019; 6: 164-75.
- Shultz SR, MacFabe DF, Ossenkopp KP, Scratch S, Whelan J, Taylor R, Cain DP. Intracerebroventricular injection of propionic acid, an enteric bacterial metabolic end-product, impairs social behavior in the rat: Implications for an animal model of autism. *Neuropharmacology*. 2008; 54: 901-11.
- Silverman JL, Yang M, Lord C, Crawley JN. Behavioural phenotyping assays for mouse models of autism. *Nat Rev Neurosci*. 2010; 11: 490-502.
- Tang G, Gutierrez Rios P, Kuo SH, Akman HO, Rosoklija G, Tanji K, Dwork A, Schon EA, DiMauro S, Goldman J, Sulzer D. Mitochondrial abnormalities in temporal lobe of autistic brain. *Neurobiol Dis*. 2013; 54: 349-61.
- Tiwari A, Khera R, Rahi S, Mehan S, Makeen HA, Khormi YH, Rehman MU, Khan A. Neuroprotective effect of α -mangostin in the ameliorating propionic acid-induced experimental model of autism in wistar rats. *Brain Sci*. 2021; 11: 1-23.
- Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol*. 2005; 57: 304.
- Wakatsuki A, Okatani Y, Ikenoue N, Fukaya T. Effect of medroxyprogesterone acetate on vascular inflammatory markers in postmenopausal women receiving estrogen. *Circulation* 2002; 105: 1436-39.
- Whitton PS. Inflammation as a causative factor in the aetiology of Parkinson's disease. *Br J Pharmacol*. 2007; 150: 963-76.

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