RESEARCH PAPER

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Effects of varied-intensity endurance exercise training on oxidative and antioxidant factors in the liver of rats with valproic acid-induced autism

Farzad Mirzavandi[1#], Nazanin Sabet[2#], Azadeh Aminzadeh[1], Mahmoodreza Heidari[1], Fatemeh Pouya[3], Amirhossein Moslemizadeh[4], Ali Saeidpour Parizi[5], Hamideh Bashiri[6]*

[1] Department of Toxicology and Pharmacology, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran
[2] Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran
[3] Department of Anatomy, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran
[4] Department of Immunology, Tehran University of Medical Sciences, Tehran, Iran
[5] Gastroenterology and Hepatology Research Center, Kerman University of Medical Sciences, Kerman, Iran
[6] Neuroscience Research Center, Institute of Neuropharmacology, Department of Physiology and Pharmacology, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran
[#]Farzad Mirzavandi and Nazanin Sabet contributed equally
**Email: h.bashiri@kmu.ac.ir, bashiry_h@yahoo.com*

Autism spectrum disorders are complex behavioral disorders that can be caused by exposure to valproic acid (VPA) during pregnancy. A therapeutic role for exercise training has been reported in many neurological diseases and problems, including autism. We aimed to evaluate various intensities of endurance exercise training and investigate its effects on oxidative and antioxidant factors in the liver of young males in a rat model of autism. Female rats were divided into a treatment (autism) and a control group. The autism group received VPA intraperitoneally on day 12.5 of pregnancy and the control pregnant females received saline. On the 30[th] day post-birth, a social interaction test was performed on the offspring to confirm autistic-like behavior. Offspring were divided into three subgroups: no exercise, mild exercise training, and moderate exercise training. Then the oxidative index of malondialdehyde (MDA) and the antioxidant indices of superoxide dismutase (SOD), total antioxidant capacity (TAC), and catalase in liver tissue were examined. The results of this study showed that both indices of sociability and social novelty decreased in the autism group. MDA levels in the liver of the autistic group increased, and moderate exercise training was shown to reduce the levels. Catalase and SOD activity as well as TAC levels decreased in the autism group, and moderate-intensity exercise training was shown to increase the values. Parameters of hepatic oxidative stress were altered in VPA-induced autism, and moderate-intensity endurance exercise training was demonstrated to have beneficial effects on hepatic oxidative stress factors by modulating the antioxidant/oxidant ratio.

Key words: autism, valproic acid, catalase, malondialdehyde, superoxide dismutase, total antioxidant capacity

INTRODUCTION

Autism spectrum disorders (ASDs) include complex behavioral disorders resulting from defects in the development of the nervous system (Sabet et al., 2021; Alinaghi Langari et al., 2022). This group of disorders has garnered significant public attention due to their high prevalence and treatment costs and their devastating impact on families (Hedley et al., 2017). Autism is a disorder that appears in the first three years of life. The disease is characterized by extensive damage to social communication, deficits in verbal and nonver-

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bal communication, uniform and repetitive patterns of behavior, and changes in pain threshold (Silvestrin et al., 2013). Other behavioral disorders that have been observed include self-harm, hyperactivity, and inappropriate sensitivity to sensory stimuli (Silvestrin et al., 2013). Environmental factors such as exposure to valproic acid may contribute to the development of autism (Herbert, 2010). Widespread use of valproic acid (VPA) for the prevention and treatment of epilepsy in pregnant women increased the risk of their children developing autism-like behavioral disorders by 81% (Lee et al., 2016).

One possible mechanism of VPA exposure-induced autism may involve the production of oxidative stress resulting in disorders of the central nervous system. Studies have shown that oxidative stress plays an important role in the development of many neurological and behavioral changes in autism (Radavi-Asgar et al., 2022).

Liver disorders are a significant health concern and are an issue commonly found in autistic children. Activation of norepinephrine-containing terminals results in inflammatory effects due to increased production of chemokines in the liver and other organs. The central nervous system damage plays a major role in the acute phase response by increasing the production of hepatic chemokines (Catania et al., 2009). Studies have shown that activation of sympathetic nerve terminals in autism can cause inflammatory responses in peripheral organs. Recent data suggest that phagocytic cells are the source of catecholamines (Flierl et al., 2007) and suppression of Kupffer cell activity after acute brain injury prevents secondary damage (Soltani et al., 2022).

Liver damage is generally assessed using histological analysis, e.g., H&E staining and liver function parameters such as glutathione (GSH), malondialdehyde (MDA), and superoxide dismutase (SOD) (Kang et al., 2014). Previous studies have shown that MDA is an indicator of lipid peroxidation as it increases following liver damage (Bakan et al., 2002) while two antioxidant enzymes, glutathione peroxidase (GPX) and SOD, decrease (Baydaş et al., 2001; Rapoport, 2012; Sabet et al., 2021). Furthermore, it has been demonstrated that the ratio of SOD to CAT was reduced in a rat model of autism (Bambini-Junior et al., 2011).

The therapeutic role of exercise training has been reported in many neurological diseases, including autism (Andoh et al., 2019). Findings show that exercise training has effects on brain activity through various mechanisms, such as increased neurogenesis, mood enhancement, and changes in neurotransmitter release (Andoh et al., 2019). Intense exercise training leads to increased oxidative stress, but regular and moderate exercise training reduces oxidative stress by increasing antioxidant defense and thus decreases the symptoms of autism (Toscano et al., 2018) and regular exercise training is a well-known method for preventing neurodegenerative diseases (Soltani et al., 2020). Additionally, reports have suggested that exercise training may increase angiogenesis (Toscano et al., 2018). People with autism experience altered sensory perception to a degree that most people do not understand (Robertson and Baron-Cohen, 2017). Studies have shown that this sensory perception is an additional burden for persons with autism and causes them to engage in behaviors that disturb those around them (Cassady, 2011). Exercise training is a major element in autism treatment programs (Pan, 2010). Running involves rhythmic movements that are similar to stereotyped behaviors, and may provide a self-stimulatory effect in people with autism similar to the way they clap their hands and walk on their toes (Al Awamleh and Woll, 2014). Researchers have found that exercise training can reduce repetitive behaviors, but this knowledge is often marginalized (Al Awamleh and Woll, 2014).

Several studies have shown that treadmill exercise training at different speeds has positive effects on motor and cognitive disorders in rat models of autism (Al Awamleh and Woll, 2014; Morakotsriwan et al., 2016; Andoh et al., 2019). However, to date, no comparisons have been made between mild and moderate exercise training intensity. Thus, this study was designed to investigate the effects of different intensities of endurance exercise training on oxidative and antioxidant factors in the liver in young male rats.

METHODS

Animals

Wistar rats were kept in a temperature-controlled environmental condition (21 ± 2°C) on a 12/12 h light/dark cycle. One male and two female rats weighing approximately 200–250 g were kept in cages together for 3 days with the aim of mating. After confirming pregnancy, female rats were separated from the male rats and kept in separate cages until delivery (Juybari et al., 2020; Shafaghi et al., 2022). The animals were subjected to standard laboratory conditions with adequate water and food access. Pregnant rats were inspected twice a day for birth control. VPA (Sigma-Aldrich) was dissolved as a sodium salt in 0.9% normal saline to reach a concentration of 250 mg/mL (Bambini-Junior et al., 2011) and was administered as a single intraperitoneal injection to the autism test group dams on day 12.5 of

pregnancy, i.e., embryonic day 12.5 (E12.5), at a dose of 600 mg/kg (female Wistar rats with controlled fertility cycles were mated overnight) – a well-known model for induction of autism traits in rats (Schneider et al., 2006). The control pregnant females received 1 ml/kg of normal saline intraperitoneally.

It should be noted that the female animals were kept in groups of four per cage, two weeks before mating, and were synchronized with a 12/12 h light/dark cycle. The estrous cycle stage was determined based on the cell type proportions observed in the vaginal secretion (Schneider and Przewłocki, 2005). The estrous cycle was monitored daily by taking vaginal smears and staining using the Papanicolaou method between 7:00 and 8:00 a.m. Briefly, the specimen was treated with alcohol in a graded concentration, stained with solutions including eosin-azure (EA50), orange G (OG6), and Harris hematoxylin, re-treated with alcohol in a graded concentration, and then placed in xylene. The estrous phase was determined by the presence of anucleate cornified cells using a light microscope with a 40-objective lens (Shamsi Meymandi et al., 2020). The morning when spermatozoa in the vaginal smear or vaginal plugs were observed was set as day 0 of pregnancy (Shamsi Meymandi et al., 2020).

The day of birth was considered postnatal day (PND) 0 and the newborn rats were randomly divided. They were weaned at 21 days of age and kept separated based on sex. A socialization test was performed to ensure that juvenile rats exhibited autism-like traits. After birth, one-month-old rats underwent exercise training with two different intensities and then oxidative and antioxidant factors were assessed. The animal care and behavioral experiments were conducted according to the "NIH Guide for the Care and Use of Laboratory Animals" and approved by the ethical committee of Kerman University of Medical Sciences, Iran (approval number: IR.KMU.REC.1400.084). The study protocol is also shown in Fig. 1.

Experimental groups

A total of 36 animals were used in this investigation. The two main groups, control (offspring from dam treated with saline, 1 ml/kg) and autism (offspring from dam treated with VPA, 600 mg/kg) (Juybari et al., 2020; Shamsi Meymandi et al., 2020), were divided into three subgroups (n=6): Control group: male offsprings that did not perform any exercise training; mild intensity exercise training group: after a week of adaptation, offsprings began to run on the treadmill five times a week for four weeks for 30 min a day at a speed of 8–10 m per min (Seo et al., 2013; Sabet et al., 2022b); moderate-intensity exercise training group: after a week of adaptation, offsprings began to run on the treadmill five times a week for four weeks at a speed of 20 m per min for 30 min (Bernardi et al., 2013; Sabet et al., 2022b).

Social interaction test (SIT) to assess social behavior

On the day of the experiment, the rat was socially isolated for 3 h to increase the animal's interest in social exploration. This test was performed in a dimly lit room using a large cage with dimensions of 260 × 420 × 180 cm, which was divided into three chambers. These chambers were connected, and the two side chambers contained an empty wire cage. During the test, three stages of habituation, socialization, and social preference were implemented and recorded.

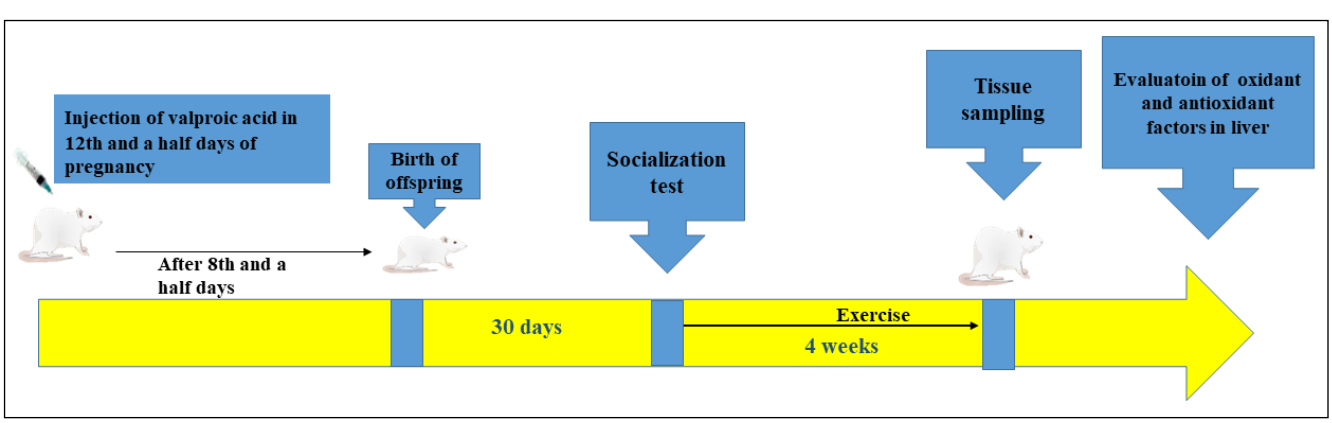

Fig. 1. A schematic representation of the experimental protocol.

Habituation stage: The rat was placed in the central chamber and free to enter and explore the other two chambers for 5 min.

Sociability stage: This stage involved placing the test rat in the center chamber, a novel animal (new rat 1) in the right or left wire cage (randomly), and recording the time spent by the animal in each part of the apparatus. In this step, we calculated the socialization index (SI) by dividing the time spent in the section with new rat 1 by the total time spent in the chamber (Shahrbabaki et al., 2022).

Social novelty stage: This step was performed ten minutes after the previous step. While the test rat was in the central chamber, another novel rat (new rat 2) was placed into the third chamber, which was previously empty and opposite the chamber of new rat 1 in the previous step. However, new rat 1 (now, the familiar rat) was not released from the cage. At this stage, the time spent by the test animal in each chamber was recorded. The social novelty index (SNI) was calculated by dividing the time spent in the section near new rat 2 by the time spent in the section near new rat 1, i.e., the familiar rat (Schneider et al., 2006; Dehghanian et al., 2022).

Evaluation of malondialdehyde (MDA) levels

MDA is a product of membrane lipid peroxidation and a biomarker of oxidative P<0.001 and can be measured by the thiobarbituric acid (TBA) method. At the end of the behavioral testing, after anesthetizing the animals with pentobarbital 50 mg/kg, the liver was rapidly removed and immediately frozen in liquid nitrogen. To measure MDA, a reaction mixture containing TBA, sodium dodecyl sulfate (SDS), 20% acetic acid (pH=3.5), and distilled water was added to the homogenized liver tissue. The resulting mixture was heated at 90°C for 45 min and, after cooling at room temperature, was centrifuged at 10,000 g for 10 min to obtain a smooth solution. Then the adsorption of the supernatant was recorded at 532 nm. The amount of lipid peroxidation was determined using a standard curve and expressed in µmol/mg of tissue (Velayutham et al., 2013; Moslemizadeh et al., 2022; Raji-Amirhasani et al., 2022).

Measurement of total antioxidant capacity (TAC)

TAC was evaluated by ferric reducing antioxidant power (FRAP) (Radavi-Asgar et al., 2022). For this purpose, homogenized samples of liver tissue were first centrifuged at 10,000 g for 7 min. The supernatant was removed from the precipitate and diluted with distilled water 5 times and then quickly used to measure antioxidants. To measure the antioxidant activity, a FRAP solution including sodium acetate, TPTZ (2,4,6-tri-pyridyl-s-3,6-triazine), and ferric chloride was used. Then, from the homogenized diluted samples, distilled water (as a control) and a standard solution with predetermined dilutions (ferrous sulfate with concentrations of 125, 250, 500, and 1,000 µM) were added to each test tube containing FRAP and were heated at 37°C in a hot water bath for 5 min. After that, the absorbance of the samples was assessed at a wavelength of 593 nm (Velayutham et al., 2013; Juybari et al., 2020).

Evaluation of catalase activity

Liver samples were homogenized in phosphate buffer (M 0.05, pH=7) and centrifuged at 7000 rpm at 4°C for 2 min. The supernatant was used for the evaluation. To a certain volume of tissue extract, potassium phosphate buffer, and oxygenated water were added and the absorption rate was read at 240 nm over 1 min (every 15 s). The absorption reduction rate was calculated per minute and the enzyme activity was expressed in U/g protein (Soltani et al., 2022; Sabet et al., 2022a).

Measurement of superoxide dismutase (SOD) activity

A certain volume of liver tissue was homogenized in saline phosphate buffer. To the homogenized tissue, EDTA (0.1 mM) in 0.3 M sodium cyanide and 75 mM nitro blue tetrazolium (NBT) were added in one cuvette and mixed for 5 to 8 min at 37°C. Then, 13 mM riboflavin and methionine were added to 0.67 M phosphate buffer (pH=7.8) and the volume was increased to 3 mL for 10 min at room temperature and in the light. The absorbance was read at 560 nm. Enzyme activity was calculated as units per mg of sample protein (Giannopolitis and Ries, 1977).

Statistical analysis

Data were expressed as mean ± SEM. The Shapiro-W test was used to assess data normality. Student's t-test was used to compare the control and autism groups in the social interaction test for induction of autism-like behavior. One-way ANOVA with a Tukey *post hoc* test was used to compare the oxidative stress variables between the groups. The level of significance was P≤0.05. GraphPad Prism software was used for data analyses.

RESULTS

The effect of VPA injection on the sociability index and social novelty index in rats for confirming autism-like traits

Fig. 2 shows the effect of injecting a dam with VPA (dose of 600 mg/kg on day 12.5 of pregnancy) on the rat's offspring's sociability index (Fig. 2A) and social novelty index (Fig. 2B) and the confirmation of autism-like traits in these animals. After statistical analysis, it was found that there was a significant difference in both indices for the autism group compared to the control group (P<0.001); this is significant as one of the primary symptoms in humans with autism is disordered social behavior.

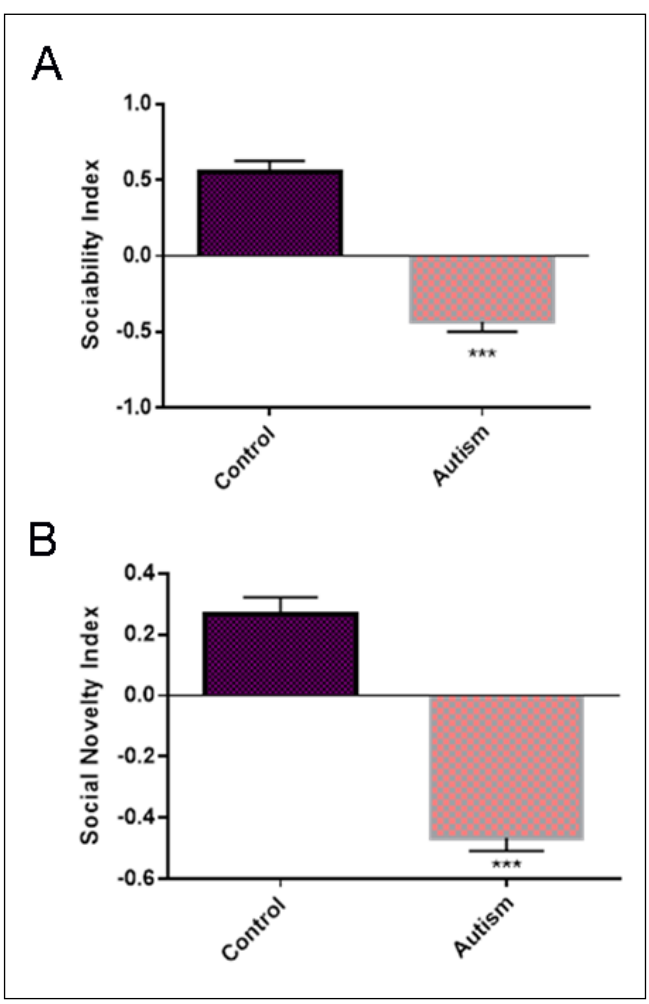

Fig. 2. Effect of VPA injection at a dose of 600 mg/kg on sociability index (A) and social novelty index (B) in rats and confirmation of autism-like traits in these animals. In each group, six animals were studied. Each column represents the mean ± SEM. *** denotes a significant difference with the control group, P<0.001.

The effect of mild and moderate endurance exercise training on MDA in liver tissue of rats with VPA-induced autism traits

Fig. 3 shows the effects of mild and moderate endurance exercise training on MDA levels in the liver tissue from a VPA-induced autism rat model. One-way ANOVA showed a significant difference between study groups for the level of MDA in liver tissue ($F_{(5,30)}$=72.39, P<0.001). Further analysis using the Tukey test revealed that MDA levels in liver tissue from the autism group were significantly higher than in the control group (P<0.001). The moderate exercise training + autism group displayed a considerably reduced MDA level compared to the autism, moderate exercise training, and mild exercise training + autism groups (P<0.001), indicating the beneficial effect of this type of exercise training on MDA modulation. In the exercise training group with mild intensity + autism, MDA levels significantly increased compared to the similar group without autism (P<0.001).

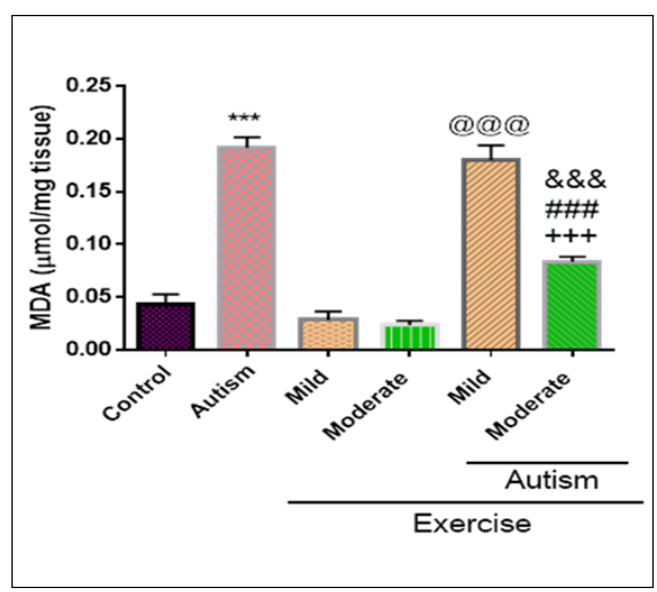

Fig. 3. Effect of mild and moderate endurance exercise training on MDA levels in the liver tissue of VPA-exposed rats. Each column represents the mean ± SEM (n=6 per group). *** denotes a significant difference with the control group, P<0.001; +++ denotes a significant difference with the autism group, P<0.001; 95 denotes a significant difference with the mild exercise training group, P<0.001; &&& denotes a significant difference with the moderate intensity exercise training group, P<0.001; and ### denotes a significant difference with the mild intensity + autism group, P<0.001.

The effect of mild and moderate endurance exercise training on catalase (CAT) activity in liver tissue from rats with VPA-induced autism traits

Fig. 4 demonstrates the effects of mild and moderate endurance exercise training on catalase activity in the liver tissue from a VPA-induced autism rat model. One-way ANOVA showed a significant difference between study groups for CAT activity in liver tissue ($F_{(5,30)}$=41.84, P<0.001). Further analysis using the Tukey test showed that CAT activity in the liver tissue from the autism group was significantly lower than in the control group (P<0.001). The moderate exercise training + autism group displayed dramatically increased CAT activity compared to the autism (P<0.05), moderate exercise training (P<0.001), and mild exercise training + autism (P<0.01) groups, suggesting this kind of exercise training beneficially modulated CAT in the autism group animals. In the mild intensity + autism group, CAT activity significantly decreased compared to the similar group without autism (P<0.001).

The effect of mild and moderate endurance exercise training on superoxide dismutase (SOD) activity in liver tissue from rats with VPA-induced autism traits

Fig. 5 shows the effects of mild and moderate endurance exercise training on SOD activity in liver tissue from a VPA-induced autism model rat. One-way ANOVA showed a significant difference between study groups in SOD activity in liver tissue ($F_{(5,30)}$=28.69, P<0.001). The Tukey *post hoc* test indicated that SOD activity in the liver tissue was significantly lower in the autism group than in the control group (P<0.001). In animals that underwent moderate exercise training, SOD activity increased compared to the control (P<0.05) and mild (P<0.01) groups. SOD activity in the moderate + autism group increased considerably compared to the autism (P<0.01) and mild + autism (P<0.05) groups, and also decreased significantly compared to the moderate group (P<0.001). In the mild + autism group, SOD activity decreased significantly compared to the mild group without autism (P<0.001).

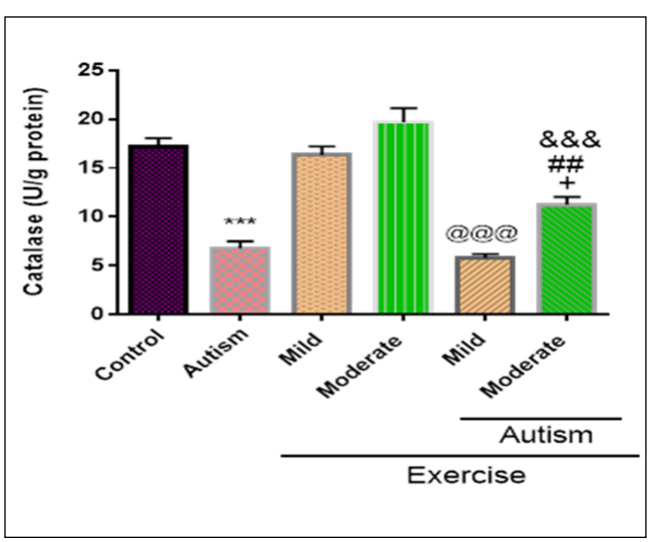

Fig. 4. The effect of mild and moderate endurance exercise training on catalase (CAT) activity in liver tissue of VPA-exposed rats. There were six animals per group. Each column represents the mean ± SEM. *** denotes a significant difference with the control group, P <0.001; + denotes a significant difference with the autism group, P<0.05; 95 denotes a significant difference with the mild exercise training group, P<0.001; &&& denotes a significant difference with the moderate intensity exercise training group, P<0.001; and ## denotes a significant difference with the mild exercise training + autism group, P<0.01.

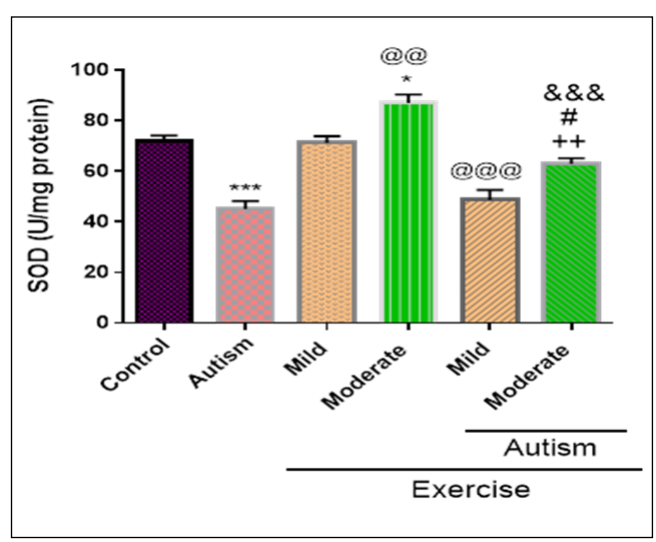

Fig. 5. The effect of mild and moderate endurance exercise training on superoxide dismutase (SOD) activity in liver tissue of VPA-exposed rats. There were six animals per group. Each column represents the mean ± SEM. * and *** denote significant differences with the control group, P<0.05 and P<0.001, respectively; ++ denotes a significant difference with the autism group, P<0.01; @@ and 95 denote the significant differences with the mild intensity exercise training group, P<0.01 and P<0.001, respectively; &&& denotes a significant difference with the moderate intensity exercise training group, P<0.001; and # denotes a significant difference with the mild intensity + autism group, P <0.05.

The effect of mild and moderate endurance exercise training on total antioxidant capacity (TAC) in liver tissue from rats with VPA-induced autism traits

Fig. 6 demonstrates the effects of mild and moderate exercise training on TAC in the liver tissue of rats exposed to VPA. One-way ANOVA showed a significant difference between study groups for TAC level in liver tissue ($F_{(5,30)}$=34.42, P<0.001). Further analysis using the Tukey test showed that TAC levels in liver tissue were significantly lower in the autism group than in the control group (P<0.001). In animals that underwent moderate exercise training, the TAC level increased compared to the exercise training group with mild intensity (P<0.001). TAC levels in the moderate intensity + autism group increased dramatically compared to the autism (P<0.05) and mild exercise training + autism (P<0.001) groups and decreased significantly compared to the moderate-intensity exercise training group (P<0.001). In the exercise training group with mild intensity + autism, the TAC level was significantly reduced compared to the exercise training group with mild intensity (P<0.01).

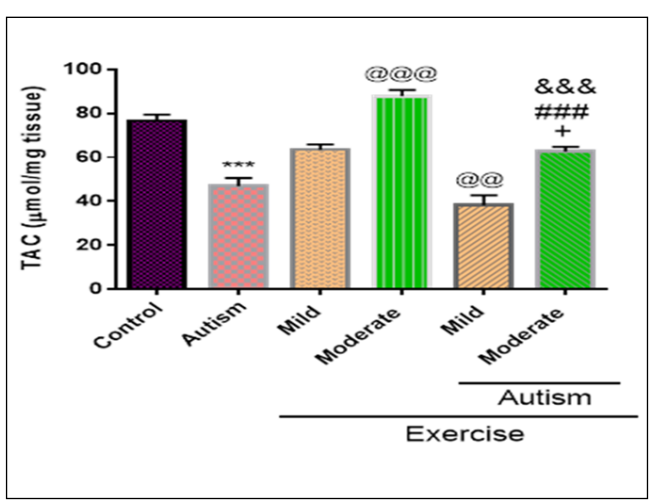

Fig. 6. The effect of mild and moderate endurance exercise training on total antioxidant capacity (TAC) in liver tissue of VPA-exposed rats. There were six animals per group. Each column represents the mean ± SEM. *** denotes a significant difference with the control group, P<0.001; + denotes a significant difference with the autism group, P<0.05; @@ and 95 denote significant differences with the mild intensity exercise training group, P<0.01 and P<0.001, respectively; &&& denotes a significant difference with the moderate intensity exercise training group, P<0.001; and ### denotes a significant difference with the mild + autism exercise training group, P<0.001.

DISCUSSION

Autism is characterized by three main types of deficits, including deficits in social interaction; impairments in language, communication, and imaginative play; and disorders in the area of interests and activities. Although autism is a neurological disease, other disorders have been reported in autism, such as cognitive impairment, depression, and changes in perception (Boyd et al., 2011; Drenthen et al., 2016). This study aimed to investigate the effects of different intensities of endurance exercise training on oxidative and antioxidant factors in the liver of young male rats.

The results of this study showed that injection of VPA at a dose of 600 mg/kg induced autism-like traits in the experimental animals, and there was a significant difference in both the sociability index and social novelty index for the autism group compared to the control group. The results of this study are in line with previous studies showing that the administration of a single dose of valproic acid on day 12.5 of pregnancy-induced autism-like traits in rats (Hirsch et al., 2020). Our previous studies also demonstrated that rats exposed to VPA prenatally present with social deficits similar to those observed in autism (Shamsi Meymandi et al., 2020; Shafaghi et al., 2022). Female rats prenatally exposed to VPA showed less reactivity to painful stimuli, less motor activity, more anxiety-like behaviors, and less caution (Shamsi Meymandi et al., 2020; Juybari et al., 2020). In male animals exposed to VPA, an increase in pain threshold and a decrease in the number of social behaviors were observed (Schneider et al., 2006). An increase in the number of stereotypical repetitive behaviors in female rats exposed to VPA may be an indicator of other stress-related mechanisms causing behavioral changes in the animals, and these should be considered and assessed in future studies. Certainly, in humans with autism, an increase in repetitive stereotypical behaviors and (Dubois et al., 2010) deficits in social interaction, as well as an increase in cortisol levels, were observed (Corbett et al., 2006).

The results of this study showed that MDA levels in liver tissue in the autism group were significantly higher than in the control group. A separate study previously showed that VPA exposure significantly increased MDA levels, compared to control rats, which is consistent with our study (Lefter et al., 2020). Additionally, the study showed that valproic acid injection can increase oxidative stress in temporal lobe tissue, which is associated with decreased antioxidant enzyme activity and high lipid peroxidation (Lefter et al., 2020). Research has also shown that

following liver damage in autism there is an increase in MDA, an indicator of lipid peroxidation (Bakan et al., 2002), and a decrease in the antioxidant enzymes GPX and SOD (Baydaş et al., 2001). The results of our study showed that MDA levels in the exercise training group with moderate intensity + autism were significantly reduced compared to the autism group and the exercise training group with mild intensity + autism.

Regular exercise has been shown to reduce oxidative stress and increase antioxidant enzymes such as superoxide dismutase (SOD), catalase and, gluilktathione peroxidase (GPX) in various organs (Sabet et al., 2022b). The results of this study also showed that liver tissue CAT levels in the autism group were significantly lower than in the control group. Oxidative stress may play an important role in this disease and stress can aggravate some symptoms of autism. Studies have shown that glutathione transferase and CAT activity levels are reduced in autism (Ghanizadeh, 2008). Consistent with our results, another study showed that the ratio of SOD to CAT decreased in rats exposed to VPA (autism group) compared to the control group (Bambini-Junior et al., 2011). In fact, the increase in the anti-oxidative enzymes may be a compensatory response to oxidative stress to inhibit free radicals (Pieper et al., 1995). The results of our study showed that the level of SOD in liver tissue was significantly lower in the autism group than in the control group. SODs are metalloenzymes that play an important role in the antioxidant defense against oxidative stress in the body. Thus, SOD may activate endogenous antioxidant systems to neutralize free radicals and act in a variety of diseases (Rosa et al., 2021).

Aerobic training improves oxidative stress in cells and tissues. There is evidence that exercise training specifically promotes the upregulation of antioxidant defenses by elevating the activity of CAT and TAC (Sabet et al., 2022b). A separate study showed that regular exercise training decreased oxidative stress and promoted the upregulation of antioxidant factors by increasing the activity of SOD and CAT (Ennezat et al., 2001). We showed that SOD levels increased slightly in animals with moderate exercise training compared to control and exercise training groups. Another study found that SOD levels decreased in rats with exercise training, although this was not the case for females (Antioch et al., 2017). One reason for the decrease in SOD levels in this study is excessive exercise training, which increases the level of oxidants in animals. However, meta-analyses in this area of research clearly show that exercise training interventions led to an improvement in the pathophysiology of autism (Sarabzadeh et al., 2019).

CONCLUSION

The results of this study demonstrate that the administration of VPA to rats on day 12.5 of pregnancy caused social deficits in the rat offspring. Additionally, parameters of hepatic oxidative stress in the VPA-induced autism model shifted, and there was a significant increase in MDA, as well as a decrease in TAC, SOD, and CAT activity. Lastly, the study showed that moderate endurance exercise training could have beneficial effects on modulating hepatic oxidative stress factors in the context of autism by increasing antioxidant indices and decreasing the oxidant index of MDA.

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