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Sex dependent alterations of resveratrol on social behaviors and nociceptive reactivity in VPA-induced autistic-like model in rats

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A B S T R A C T

Introduction: The present study was designed to clarify the effects of resveratrol (RSV) on social behavioral alterations and nociceptive reactivity in valproic acid (VPA)-induced autistic-like model in female and male rats.
Methods: Pregnant Wistar rats were randomly divided in five groups. Animals received saline, DMSO, VPA, RSV and RSV + VPA. VPA was administered (600 mg/kg, i. p.) on embryonic day 12.5 (E12.5) and pretreatment by resveratrol (3.6 mg/kg, s. c.) was applied on E6.5 until E18.5. All offspring were weaned on postnatal day 21 and the experiments were done in male and female rats on day 60. Social interaction, hot plate and tail flick tests were set out to assess social deficits and pain threshold, respectively. Sociability index (SI), Social novelty index (SNI) and latency time were calculated as the standard indices of social behaviors and pain threshold, respectively.
Results: The results indicated that systemic intraperitoneal administration of VPA (600 mg/kg) significantly decreased SI and SNI in social interaction test (SIT) especially in male rats, indicating the social impairments caused by VPA. RSV (3.6 mg/kg, s. c.) reversed VPA-induced social deficits in male rats, but not in female group. VPA administration resulted in significant increase in latency time in the hot plate and tail flick tests in male rats, whereas it had no such dramatic effect in females. RSV administration in combination with VPA had no significant effect on latency time compared to the valproic acid group in male rats. It is important to note that RSV by itself had no significant effect on SI, SNI and latency time in female and male rats.
Conclusion: It can be concluded that valproic acid produces autistic-like behaviors and increases pain threshold in male rats which may be ameliorated at least in part by resveratrol administration. Further studies are needed to elucidate the molecular mechanisms involved in valproic acid and resveratrol-induced effects.

1. Introduction

Autism as one of the most prevalent growing neurodevelopmental disorders worldwide, comprises a group of severe and pervasive neurodevelopmental disorders with great etiological and clinical heterogeneity, characterized by compromised social interactions, language and communication impairments, stereotyped, restrictive, and repetitive interests, behaviors, or activities (Miles, 2011; Schneider et al., 2008). The clinical findings demonstrate aberrant sensitivity to sensory stimulation in individuals with autism spectrum syndrome

Abbreviations: ASD, autism spectrum syndrome; BLA, basolateral amygdala; CBR1, carbonyl reductase 1; GABA, ɣ-aminobutyric acid; GSTP1, glutathione S-transferase P; NAc, nucleus accumbens; NQO2, NAD(*P*)H dehydrogenase quinone 2; PFC, prefrontal cortex,; RSV, resveratrol; VPA, valproic acid; SI, social interaction; SNI, Social novelty test; SIT, social interaction test; HPT, hot plate test; TFT, tail flick test

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(ASD). The prevalence of sensory processing impairments and abnormalities in cognitive processes ranged from 69% to 95%, among autistic children. On the whole, findings about behavioral responses are inconsistent and hyposensitivity, hypersensitivity, or both can be exhibited in the children with autism (Yasuda et al., 2016; Baranek et al., 2006).

According to reviews conducted by some authors, autistic individuals show discrepancies in subjective experience of pain (i.e. different pain thresholds) and differences in behavioral reaction in response to a painful stimulus. (Moore, 2015; Allely, 2013).Some prevailing reports considered that children with autism have either reduced or increased sensitivity to pain (Bursch et al., 2004; Mieres et al., 2011). In contrast, other studies display a substantial behavioral response to nociceptive stimuli in autistic children (Nader et al., 2004; Daughters et al., 2007). The findings from the majority of studies investigating pain in children and adults with ASD indicated that impaired sensory perceptions can alter or delay the response to various types or degrees of pain. Furthermore, individuals with autism do not respond to pain in the same way that children without autism do. These reasons can mistakenly direct parents, caregivers and mental health professionals to this concept that the child is in no pain (Allely, 2013).

A wide range of environmental risk factors such as prenatal exposure to agents e.g. valproic acid (VPA) as a broadly used medicine in epilepsy therapy play a critical role in altered brain development leading to ASD. Animal studies have shown a notable incidence rate of autistic alterations among rats gestationally exposed to VPA (Prandota, 2010), (Ornoy, 2009). VPA exposure during a special period of pregnancy can cause morphological and functional impairments in fetus such as defect of closing neural tube during neuro-developmental process and also behavioral disorders. Previous clinical and animal studies have shown that in utero VPA exposure during the first trimester of pregnancy strongly increases the risk of ASD in newborn infants (Viinikainen et al., 2006; Williams et al., 2001; Wagner et al., 2006; Arndt et al., 2005). Several lines of evidence suggest that some behavioral alterations of ASD induced by VPA can be explained by abnormalities in various brain regions. Hara et al. (2012) reported that VPA exposure at embryonic day 12.5 caused Nissl-positive cell loss in prefrontal cortex (PFC) in both male and female mice and in somatosensory cortices in male but not females. These findings proposed that VPA-induced morphological abnormalities in the somatosensory cortex are probably involved in the sex-dependent social interaction deficits in autistic animals (Hara et al., 2012). Prenatal VPA exposure may produce changes in the cortical thickness at level of the PFC, basolateral amygdala (BLA), and hippocampus with implication in patients with the ASD (Sosa-Díaz et al., 2014). In addition, it has been shown that VPA-exposed rats increased expression and activity of γ-aminobutyric acid (GABA) transporters in the amygdala and decreased GABA synthesis in the cerebellum, which was manifested as an increase in anxiety-like behaviors (Olexová et al., 2016). Neurochemical and electrophysiological alterations in the nucleus accumbens (NAc) may also contribute to the inability of VPA-exposed rats to process and respond to social cues (Schiavi et al., 2019). Using clinical studies, it has been demonstrated that several brain features have been involved during ASD including cortical surface area growth, increased volume of extra-axial CSF, aberrant white matter connectivity and altered functional connectivity which is associated with behavioral symptoms such as early motor deficits, stereotyped and repetitive behaviors and sensory responsiveness, social and language deficits (Shen and Piven, 2017).

Resveratrol is a natural polyphenolic compound considered to have several benefits for health. This compound is found in various edible species of plants, particularly in grapes.

Resveratrol can activate many specific targets including NAD(*P*)H dehydrogenase quinone 2 (NQO2), Glutathione S-transferase P (GSTP1), estrogen receptor beta, Carbonyl reductase 1 (CBR1), integrin αVβ and sirtuin 1 (Vang, 2015), which regulate downstream pathways such as antioxidant enzymes. However, it is still not well-established which receptor is responsible for its observed effects in cells and model organisms. This low aqueous soluble agent is extensively metabolized in the body, especially in the liver and lung, and its bioavailability is about 0.5% because of extensive hepatic glucuronidation and sulfation (Higdon et al., 2016).

The finding from animal studies or clinical trials has shown that resveratrol may modulate multiple disorders including neurological nervous diseases by different mechanisms such as anti-inflammatory and antioxidant effect (Vang et al., 2011; Vázquez-Agell et al., 2007; Palsamy and Subramanian, 2010; Martín et al., 2006). According to Sharma et al. (2007) report, resveratrol in combination with other substances such as insulin significantly attenuated thermal hyperalgesia and the hot-plate latencies in streptozotocin (STZ) induced diabetic mice (Sharma et al., 2007). Furthermore, there are numerous animal studies indicating that resveratrol showed neuroprotective activity in injury or neurodegeneration (Tsai et al., 2007; Lu et al., 2006; Jin et al., 2008).

Based on (Miles, 2011) absence of successful and effective treatment for autistic patients, (Schneider et al., 2008) the evidence which shows neuroprotective effects of prenatal resveratrol administration on nervous diseases such as ASD due to antioxidant and anti-inflammatory activity (Bambini-Junior et al., 2014), and also (Yasuda et al., 2016) an approximate ratio of 4:1 for occurrence rate of autism in boys than girls, the present study set out to assess the effects of prenatal administration of RSV on autistic-like social behaviors and pain sensitivity considering potential sex differences in male and female Wistar rats exposed to VPA on gestational day12.5.

2. Materials and methods

2.1. Animals

Female Wistar rats (Institute of Medical University of Kerman), with controlled fertility cycle were mated overnight. It should be noted that the female animals were kept in groups of four per cages two weeks before mating and were synchronized with a 12-hour light/dark cycle. The evaluation of the stage of estrous cycle was randomly conducted based on the proportion of cells types observed in the vaginal secretion (Caligioni, 2009). The estrous cycle was daily monitored by taking and staining the vaginal smears with 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 haematoxylin, re-treated with alcohol in graded concentration and placed in xylene. The estrous cycle stage was specified by a light microscope with 40 objective lens. Estrous phase was determined by the presence of anucleated cornified cells (Maghool et al., 2013). The morning when spermatozoa in vaginal smear or vaginal plugs were observed was set as day 0 of pregnancy (Kusanagi, 1983; Baronio et al., 2015). Pregnant Wistar rats were randomly separated in five groups and housed in group of two per cage under temperature controlled environment (23 ± 1 °C) and 12/12 h light–dark cycle with free access to food and water as follows:

Group I. VPA group: Pregnant rats received a single intra-peritoneal injection of 600 mg/kg sodium VPA (Sigma-Aldrich) prepared in 0.9% normal saline for a concentration of 250 mg/mL (Bambini-Junior et al., 2011) on embryonic day12.5 (E12.5), as a well-known model for induction of autism in rats (Schneider et al., 2008).This dose can result in 900 μg/mL of total VPA in maternal plasma in < 1 h, with an average plasma elimination half-life of 2.3 h (Binkerd et al., 1988).

Group II. RSV group: Pregnant rats were treated daily with resveratrol (Sigma-Aldrich) (3.6 mg/kg, SC) for a concentration of 36 mg/mL from E6.5 to E18.5 (Bambini-Junior et al., 2014). Since the high doses of RSV can cause maternal behavioral pervasive

effects, the dose of 3.6 mg/kg as an equal with 250 mg of RSV to a 70 kg human, and also a very low dose to rodents was selected (Roberts et al., 2014).

Group III. VPA + RSV group: Pregnant rats were treated with a single intra-peritoneal injection of 600 mg/kg sodium VPA prepared in 0.9% normal saline on E12.5 and pretreated with resveratrol (3.6 mg/kg, SC) from E6.5 to E18.5.

Group IV-V. Vehicle groups: Females were injected with vehicles, physiological normal saline (VPA solvent) or DMSO (RSV solvent) at the same time with an equal volume of VPA or RSV, respectively. After delivery, females were housed individually and allowed to raise their own litters until day 21. To avoid litter/dam effects, one offspring from each litter was contributed to each pharmacological group. Then the offspring were housed with 2–3 rats in same-sex, same-treatment cages. Rat pups were kept in rooms under controlled humidity, temperature at 23 ± 1 °C and 12 h light–dark cycles (lights on at 8 a.m., lights off at 8 p.m.) with free access to food (standard laboratory pellets) and water. All the procedures were conducted in accordance with the regular ethical guidelines (NIH, publication no. 85-23, revised 1985; European Communities Directive 86/609/EEC) and approved by the ethical committee of Kerman University of Medical Sciences, Kerman, Iran (Ethical number knrc/95-61). Three-chamber social interaction test was applied to assess the social behaviors, and nociceptive effects were evaluated using hotplate and tail flick tests. The basic time course of the study has been demonstrated in Fig. 1. These tests were performed at 56–60 days after birth. In this step, all the experiments were carried out in the light phase between 09:00 a.m. and 14:00 p.m.

2.2. Three-chamber sociability and social novelty test

The test was carried out under dim light/unfamiliar conditions as explained previously (Sargin et al., 2011). The three-chambered apparatus (Behboud Tahghigh Kerman) is based on rodent nature to explore a novel context. Testing follows three sessions:

2.2.1. Habituation period

The subject rat was placed at the center of the middle chamber and allowed to explore for five minutes.

2.2.2. Sociability period

After the habituation period, an unfamiliar male (stranger 1), that had no prior contact with the subject rat, was placed inside a small, round wire cage in one of the side chambers. The placement of stranger 1 in the left or right side of the chamber was systematically interchanged between trials. Both doorways to the side chambers were then opened and the subject rat was allowed free access to the entire social test box for about 10 min. The time spent in each chamber and the duration of active interaction of the subject animal with the small, round wire cage containing or not containing the stranger 1, was monitored and recorded by two trained human observers. An entry was determined when the head and all four paws had entered into one chamber.

2.2.3. Social novelty period

After the first 10 min trial, each rat was further tested in a second 10-min session to evaluate social behaviors for a new stranger. A second, unfamiliar rat (stranger 2) was placed and enclosed in the previously empty small, round wire cage in the opposite side chamber. The subject rat had a choice between the first, already explored rat (familiar stranger 1), and the novel unfamiliar rat (new stranger 2). The same parameters as described above, the duration spent in each chamber and the number of transitions between chambers of the apparatus were measured during the second 10 min session. Based on the duration spent in each chamber and the duration of active interaction based on sniffing total time, a 'sociability index' (SI) and a 'social novelty index' (SNI) (with a value of 0 meaning no preference) were assessed as follows:

$$SI = \frac{Time\ exploring_{novel\ rat\ 1} - Time\ exploring_{novel\ object}}{Time\ exploring_{novel\ rat\ 1} + Time\ exploring_{novel\ object}}$$

$$SNI = \frac{Time\ exploring_{novel\ rat\ 2} - Time\ exploring_{known\ rat}}{Time\ exploring_{novel\ rat\ 2} + Time\ exploring_{known\ rat}}$$

$$SI\ (Chamber\ time) = \frac{Time\ spent\ in\ chamber\ (_{novel\ rat\ 1}) - Time\ spent\ in\ chamber\ (_{novel\ object})}{Time\ spent\ in\ chamber\ (_{novel\ rat\ 1}) + Time\ spent\ in\ chamber\ (_{novel\ object}).}$$

$$SNI\ (Chamber\ time) = \frac{Time\ spent\ in\ chamber\ (_{novel\ rat\ 2}) - Time\ spent\ in\ chamber\ (_{Known\ rat})}{Time\ spent\ in\ chamber\ (_{novel\ rat\ 2}) + Time\ spent\ in\ chamber\ (_{Known\ rat})}$$

2.3. Tail flick test

The rat was lightly restrained with the tail placed in the groove of tail flick instrument (Borj Sanat Azma). The distal third of its tail is exposed to an intense light beam. This test was used to determine the pain threshold. The latency for the rat to flick its tail was calculated automatically by the instrument. To avoid any damage to animals, the light was automatically turned off after 20 s (Cut off = 20 s). The test was performed three times for each animal with 60 s intervals between trials, and the average of three times considered as the latency performance.

2.4. Hot-plate test

This test is also used to measure pain sensitivity. The device consists of a 19 cm diameter plate and a plexi glass wall at a height of 30 cm. This device, which is heated by electrical resistance, is connected to the timer and thermostat. The rat was placed on the surface of the described device, which was kept at a constant temperature of 55 °C. The

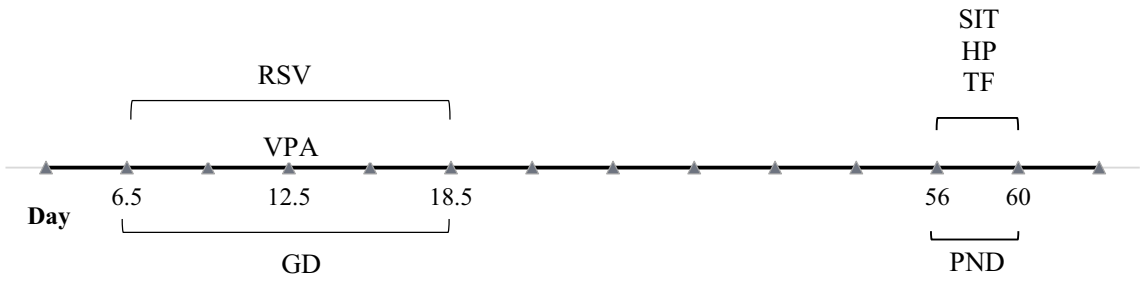

Fig. 1. The basic time course of the study. RSV, Resveratrol; VPA, Valproic acid, SIT, Social interaction test; HP, Hot plate; TF, Tail flick, GD, Gestational day; PND; Postnatal day.

latency to their first behavior such as licking, jumping, shaking, or lifting of the hind paw) was recorded by an observer. The maximum reaction time for each animal was considered to be 30 s to prevent any damage to animal (cut off = 30 s). The test was performed three times for each animal, and the average of three times was considered as the latency performance.

2.5. Statistical analysis

Data were presented as mean ± SEM. Statistical significance was analyzed using one-way analysis of variance (ANOVA), followed by the Tukey HSD post hoc test. All analyses were conducted using Sigma Stat software version 3.1 (SPSS Inc., Chicago, IL) and GraphPad Prism version 4.00, (GraphPad Software, San Diego, USA). Statistical values at $P \leq .05$ were considered significant.

3. Results

3.1. The effect of resveratrol on sociability index (SI) derived from active interaction (sniffing) in SIT in VPA-induced autistic-like model in rats

Fig. 2 illustrates the effect of RSV, VPA and VPA + RSV with the aforementioned doses on SI derived from sniffing in male (Fig. 2A) and female (Fig. 2B) rats. One-way analysis of variance showed that administration of VPA and RSV had significant effects on SI of male rats in different experimental groups [$F_{(4,30)} = 3.14$, $p < .05$], as well as female rats [$F_{(4,35)} = 8.15$, $p < .001$]. Tukey's analysis revealed that the administration of VPA in male and female rats significantly reduced SI in comparison with saline group ($p < .05$ and $p < .01$, respectively) (Fig. 2A and B). Whereas, no significant difference was found in any of the two genders compared to the DMSO group when RSV was administrated alone ($p > .05$) (Fig. 2A & B). Moreover, SI had a significant increase in male rats in the RSV + VPA group compared to the VPA group ($p < .05$) (Fig. 2A). Conversely, in female rats, administration of RSV in companion with VPA significantly decreased SI compared to the VPA group ($p < .05$) (Fig. 2B).

The effect of resveratrol on social novelty index (SNI) derived from active interaction (sniffing) in SIT in VPA-induced autistic-like model in rats

Fig. 3 shows the effect of RSV, VPA and VPA + RSV on SNI derived from sniffing in male (Fig. 3A) and female (Fig. 3B) rats. One-way ANOVA demonstrated that administration of VPA and RSV could have significant effects on the SNI of male rats in different experimental groups [$F_{(4.30)} = 9.27$, $p < .001$], without any significant effect [F (Baranek et al., 2006; Kusanagi, 1983) = 0.66, $p > .05$] on the SNI of female rats in different experimental groups. Following Tukey's analysis in male rats showed that the administration of VPA significantly decreased SNI parameter compared to the saline group ($p < .01$) (Fig. 3A). No significant difference was found in RSV group compared to the DMSO group ($p > .05$) (Fig. 3A). Moreover, SNI of male rats in the RSV + VPA group significantly increased compared to the VPA group ($p < .05$) (Fig. 3A).

3.2. The effect of resveratrol on SI derived from time spent to chambers in SIT in VPA-induced autistic-like model in rats

The effect of VPA, RSV, and VPA + RSV on index of SI based on duration spent to each chamber through the SIT in male and female rats has been shown in Fig. 4A and B, respectively. One-way analysis of variance showed that administration of VPA and RSV could have significant effects on SI of male rats in different experimental groups [F (Baranek et al., 2006; Lu et al., 2006) = 6.03, $p < .001$], as well as female rats [F (Baranek et al., 2006; Kusanagi, 1983) = 2.6, $p = .05$]. Following Tukey's analysis revealed that the administration of VPA in male rats significantly reduced SI in comparison with saline group ($p < .01$) (Fig. 4A), while this parameter did not show significant difference between these two groups in female rats (Fig. 4B). Whereas, no significant difference was found in any of the two genders compared to the DMSO group when RSV administrated alone ($p > .05$) (Fig. 4A & B). Moreover, SI had a significant increase in male rats in the RSV + VPA group compared to the VPA group ($p < .001$) (Fig. 4A). Contrary, in female rats, administration of RSV in companion with VPA could not have significant effect (Fig. 4B).

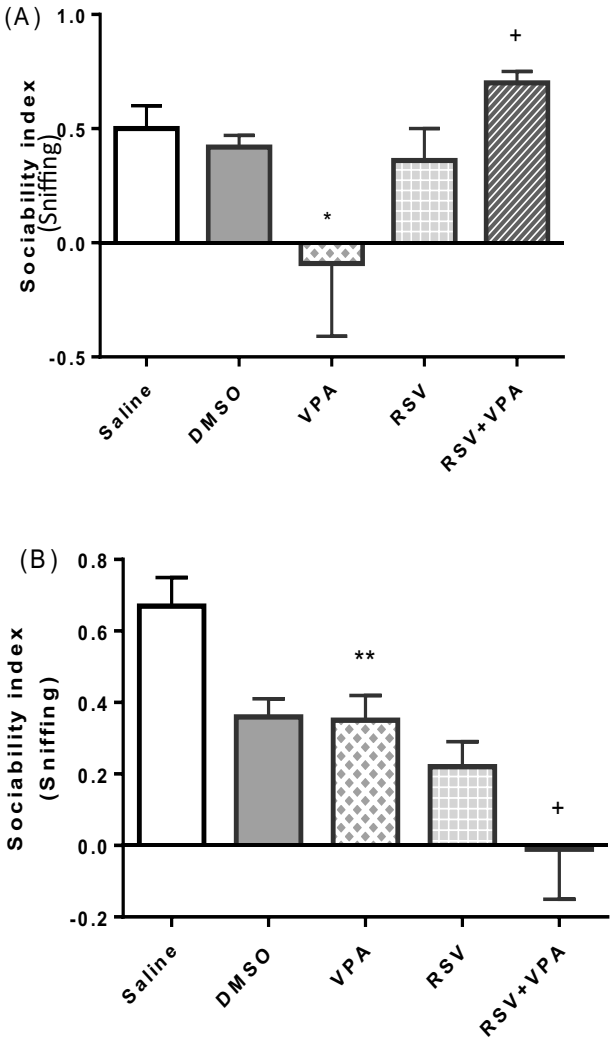

Fig. 2. The effect of VPA and RSV either alone or in combination on the sociability index (SI) derived from sniffing in male (A) and female (B) rats on day 56–58. Seven *male and eight female rats were used in each* group of *experiments*. $^{*}p < .05$ and $^{**}p < .01$ compared to the saline group, $^{+}p < .05$ compared to the VPA group. Data are expressed as mean ± SEM.

3.3. The effect of resveratrol on SNI derived from time spent to chambers in SIT in VPA-induced autistic-like model in rats

Fig. 5 illustrates the effect of VPA, RSV, and VPA + RSV on index of SNI based on duration spent to each chamber through the SIT in male (Fig. 5A) and female (Fig. 5B) rats. One-way ANOVA demonstrated that no treatment could have significant effects on the SNI of male and female rats in different experimental groups [$F_{(4.30)} = 2.24$, $p > .05$] and [$F_{(4, 35)} = 0.17$, $p > .05$], respectively.

The effect of resveratrol on the pain threshold using hot plate test in VPA-induced autistic-like model in rats

As shown in Fig. 6, the effect of VPA, RSV and VPA + RSV on pain response was assessed via hot plate test in male (6A) and female (6B) rats. One-way ANOVA showed that administration of VPA and RSV

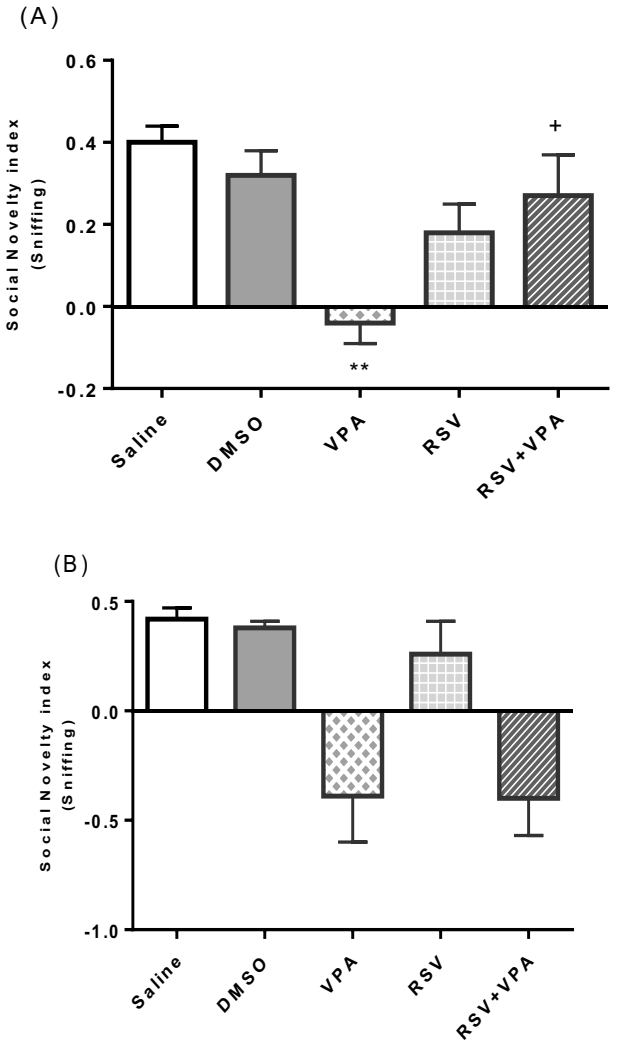

Fig. 3. The effect of VPA and RSV either alone or in combination on the social novelty index (SNI) derived from sniffing in male (A) and female (B) rats on day 56–58. Seven *male and eight female rats were used in each* group of *experiments*. $^{**}p < .01$ compared to the saline group, $^{+}p < .05$ compared to the VPA group. Data are expressed as mean ± SEM.

changed significant latency time in male rats [$F_{(4, 30)} = 4.31, p < .01$] as well as female ones [$F_{(4, 35)} = 3.76, p < .05$]. Following Tukey analysis indicated that the administration of VPA in male rats significantly increased latency time compared to the saline group ($p < .01$) (Fig. 6A), while latency time did not show significant differences between these two groups in female rats (Fig. 6B). RSV failed to produce any significant alteration in any of the two genders compared to the DMSO group when administrated alone ($p > .05$) (Fig. 6A & B). Moreover, the latency time in male rats treated with RSV + VPA showed no significant difference compared to that of the VPA group (Fig. 6A), while the latency time in female rats treated with RSV + VPA was significantly higher than that of the VPA group ($p < .05$) (Fig. 6B).

3.4. The effect of resveratrol on the pain threshold using tail flick test in VPA-induced autistic-like model in rats

The effect of VPA, RSV and VPA + RSV on pain was evaluated through tail flick test in male and female rats (Fig. 7A and B, respectively). One-way ANOVA showed that the administration of VPA and RSV exert significant effects on the latency time in male rats [$F_{(4.30)} = 6.93$, $p < .001$], without any significant effect on latency time

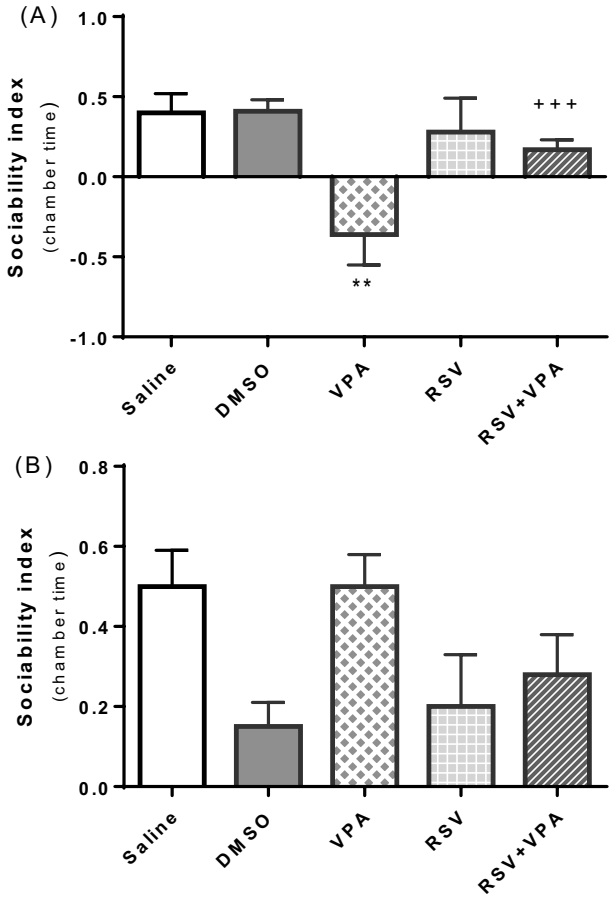

Fig. 4. The effect of VPA and RSV either alone or in combination on the sociability index (SI) derived from time spent to chambers in male (A) and female (B) rats on day 56–58. Seven *male and eight female rats were used in each* group of *experiments*. $^{**}p < .01$ compared to the saline group. $^{+++}p < .001$ compared to the VPA group Data are expressed as mean ± SEM.

in female ones [$F_{(4, 35)} = 1.47$, $p > .05$]. The results from Tukey's analysis revealed that administration of VPA significantly increased latency time in male rats compared to the saline group ($p < .01$) (Fig. 7A). RSV failed to produce any significant alteration in latency time compared to the DMSO group when administrated alone ($p > .05$) (Fig. 7 A). Moreover, the latency time in male rats treated with RSV + VPA showed significant decrease compared to that of the VPA group (Fig. 7A).

4. Discussion

The aim of the present study was to assess the effect of intraperitoneal administration of RSV on postnatal behaviors of male and female rats including alteration in social behaviors and pain threshold in a model of autism induced by prenatal exposure to VPA. The results of the study showed that intraperitoneal administration of VPA (600 mg/kg) on day 12.5 of gestation leads to autistic-like behaviors such as abnormal social interaction particularly in males. Furthermore, there was an increase in latency time of tail flick and hot plate tests in adult male rats exposed to VPA at embryonic time, but not in females.

The results of the present study were in line with previous findings indicating that male rats receiving VPA were less sensitive to pain and had more disturbance in social behaviors, while female rats did not have specific behavioral alterations in comparison with the control group (Schneider and Przewłocki, 2005). Moreover, in accordance with Schneider et al. report, our results indicated that the VPA females exhibited lower sensitivity to painful stimuli in hot plate test, compared to

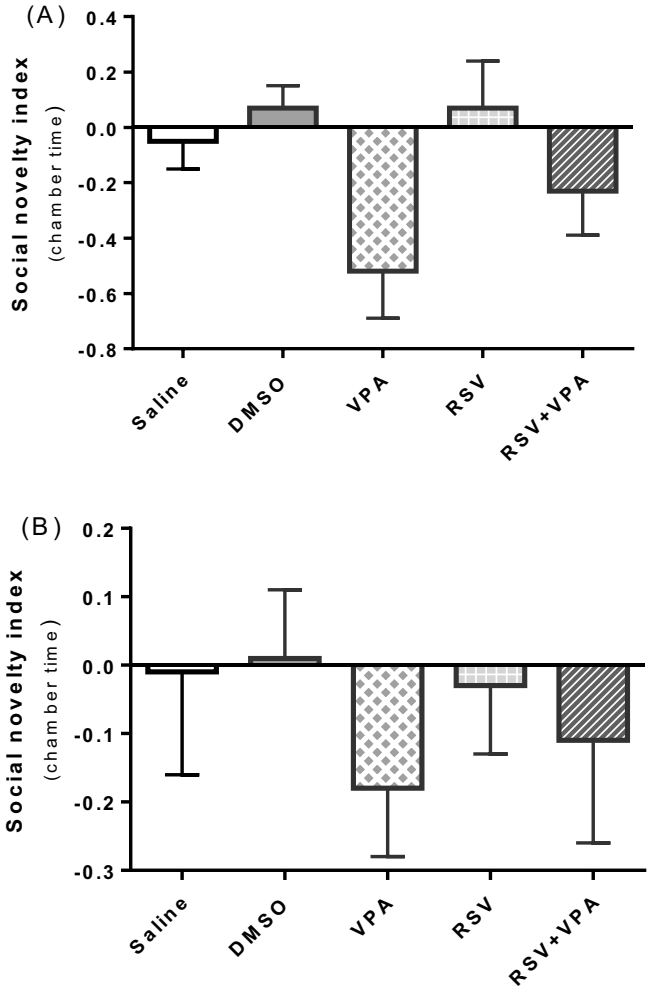

Fig. 5. The effect of VPA and RSV either alone or in combination on the social novelty index (SNI) derived from time spent to chambers in male (A) and female (B) rats on day 56–58. Seven *male and eight female rats were used in each* group of *experiments*. Data are expressed as mean ± SEM.

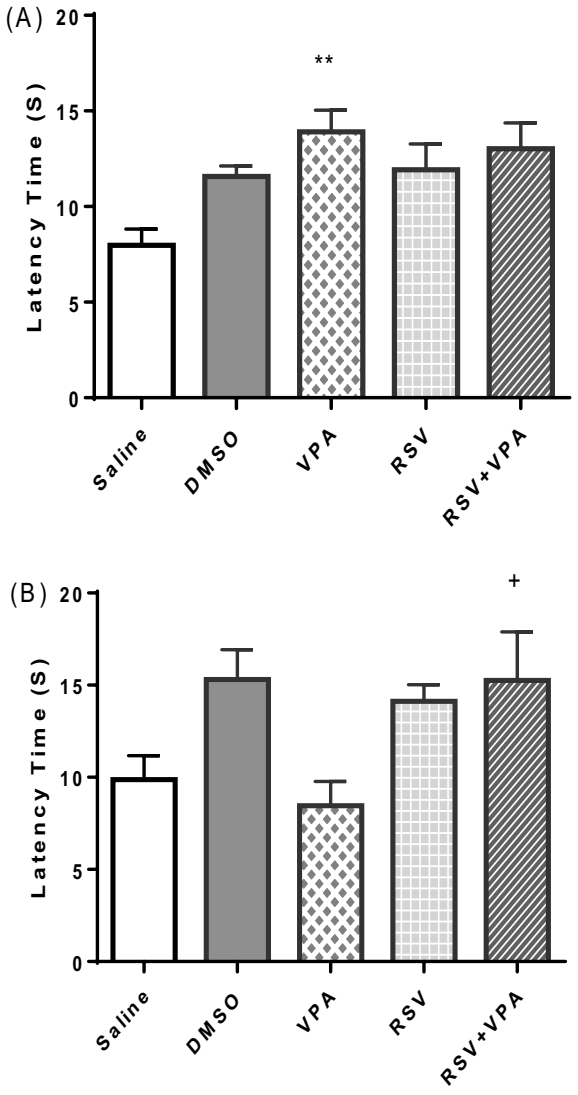

Fig. 6. The effect of VPA and RSV either alone or in combination on the pain threshold using hot plate test in male (A) and female (B) rats on day 59. Seven *male and eight female rats were used in each* group of *experiments*. $^{**}p < .01$ compared to the saline group, group, $^{+}p < .05$ compared to the VPA group. Data are expressed as mean ± SEM.

the control females (Takahashi and Kalin, 1991). On the basis of the results of previous studies, autistic-related behavioral disturbances including abnormal pain reactivity (Militerni et al., 2000), increased repetitive stereotyped behaviors (Pierce and Courchesne, 2001), high anxiety (Towbin et al., 2005) and, social communication problems may be linked to increased levels of cortisol (Corbett et al., 2006). On the other hand, in an another study it has been proposed that a single administration of VPA to pregnant rats on gestational day 12.5 yielded offspring with enhanced fear processing caused by the hypersensitivity and hyperactivity of lateral amygdala, which may be the reason of the autistic behavioral symptoms such as decreased social interactions and increased repetitive behaviors in VPA-treated offspring (Markram et al., 2008). Controversial results as the effects of VPA on nociceptive threshold and latency time and also social behaviors between male and female rats may be due to gender-related factors such as humoral factors, behavioral variables, the alteration of hypothalamic–pituitary–adrenal (HPA) axis activity and involvement of different neurotransmitters and various brain regions.

It is unclear how prenatal exposure to VPA causes behavioral and humoral alterations between male and female offspring through gender differences. However, there is evidence indicating gender differences in functional outcome of VPA treatment cannot be clarified by differences in direct VPA effect on embryos. Histological examinations obtained from brain injuries induced by VPA have not revealed any differences between males and females (Ingram et al., 2000; Rodier et al., 1996; Miranda and Toran-Allerand, 1992). Some in vitro studies have shown that VPA exerts inhibitory effects on estrogen signaling which play a critical role in brain development (Fortunati et al., 2008; Reid et al., 2005) (Miranda and Toran-Allerand, 1992). With regards to Schneider et al. (2008) report, the protective effects of estrogen and progesterone and gender-related differences in modulation of neurotransmitter release or regulation of neurotransmitter function during consecutive developmental stages can be attributed to the detected attenuation of the abnormalities induced by VPA in female rats (Schneider et al., 2008). Stein (2001) and Roof and Hall (2000) also studied the protective outcome of estrogen and progesterone on consequences of brain injuries and suggested that these gonadal hormones may have a neuroprotective effect through improvement of anti-oxidant effect and promotion of synaptogenesis. This prospect should be taken into account for future studies (Stein, 2001; Roof and Hall, 2000).

In the present study, the obtained results revealed that administration of RSV as a neuroprotective, antioxidant and anti-inflammatory agent on days 6.5 to 18 of gestation resulted in a reduction in autistic-like behaviors caused by VPA, particularly in male rats. Our data are

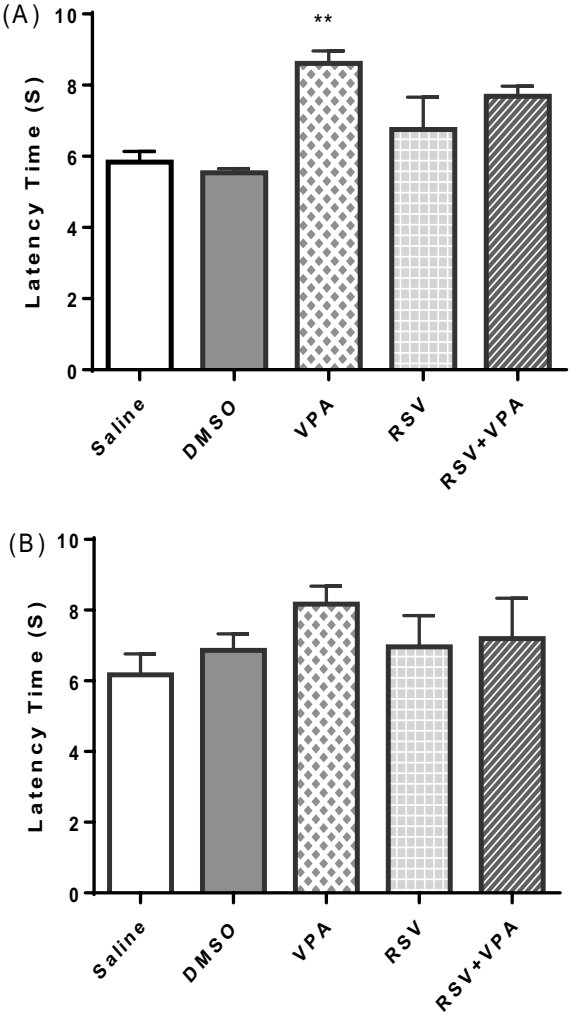

Fig. 7. The effect of VPA and RSV either alone or in combination on the pain threshold using tail flick test in male (A) and female (B) rats on day 60. Seven *male and eight female rats were used in each* group of *experiments*. $^{**}p < .01$ compared to the saline group, group, Data are expressed as mean ± SEM.

consistent with those of Bambini-Junior et al. (2014), reporting that RSV prenatal treatment in male rats could attenuate autistic-like social behaviors in a rodent model of autism induced by VPA (Bambini-Junior et al., 2014). However, they investigated the effects of RSV on VPA-induced social deficits only in male rats and did not have any sex-dependent comparison in behavior of rats. Therefore, we tried to survey and compare the effects of RSV on social behaviors and pain threshold in ASD induced by VPA in both gender. In our study, administration of RSV in companion with VPA significantly decreased SI compared to the VPA group in female rats unlike male rats. A number of studies have demonstrated that resveratrol exerts sex-dependent effects in some conditions such as DSS-induced colitis (Soylemez et al., 2008; Wagnerova et al., 2017). According to the investigations, the modulating effect of resveratrol on estrogen responsiveness may differ between male and female rats (Bursch et al., 2004; Soylemez et al., 2008). Since VPA can induce inhibitory effects on estrogen pathways (Miranda and Toran-Allerand, 1992), it can be hypothesized that discrepant effects of RSV on VPA-exposed female and male rats may depend on estrogen signaling.

Findings of the extant study demonstrated that the alteration of VPA-induced pain threshold may be modulated by resveratrol administration. In agreement with these results, a recent study performed by Marouf et al. (2018), showed that administration of RSV as adjuvant with meloxicam for 90 days led to a reduction in the pain severity in patients with mild to moderate clinical evidence of knee osteoarthritis. Furthermore, assessment of biochemical markers indicated that RSV could significantly decrease serum levels of many inflammatory mediators such as TNF-α, IL-1β compared to placebo (Marouf et al., 2018). In this respect, Tao et al. (2016) also reported that RSV has the ability to reduce neuropathic pain by adjusting the balance between pro-inflammatory and anti-inflammatory cytokines in mice (Tao et al., 2016). The study of the pathways and structures that explains these responses can be a promising topic for research on helpful ways for understanding how to modulate etiologies and molecular changes involved in the behavioral impairments in individual with autism. *Notwithstanding* the obtained results of previous studies have shown that resveratrol can prevent or reverse the nociception deficit induced by VPA in males, there was no significant effect on VPA-induced reduction in nociception in our study. It could be considered that single dose and concentration and timing of resveratrol may not have been sufficient for preventing VPA-induced nociception deficits.

5. Conclusion

It has been shown that using a single IP injection of VPA on 12.5 day of gestation, has a direct effect on induction of autistic-like behaviors in animal model of rats including social deficits and hyposensitivity to pain. Also, injection of RSV on days 6.5 to 18.5 of pregnancy could attenuate some of the sever effects of VPA in the male sex. Understanding the underlaying molecular waterfall involved in the disease and after drug treatment of RSV can guide us in the path of finding the probable ways of autism and so using effective medication therapy to cease adverse effects of autistic-like symptoms.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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