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The possible protective and therapeutic effects of Silymarin in rat model of valproic acid-induced autism

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- Silymarim
- oxidative stress
- PFC

Abstract

Autism spectrum disorder (ASD) is a complicated neurodevelopmental disorder (NDD) known by two basic symptoms: impairment in social communication and repetitive behaviors. Unfortunately, there is no definitive cure for ASD, therefore further research is needed to improve patient outcome. In our research we aimed to assess the possible protective and therapeutic effects of Silymarin on cerebellum, hippocampus and prefrontal cortex in rat model of VPA-induced autism. **Methods**: The experiment was conducted on 35 Sprague-Dawley male rats divided into five equal groups; Control group; 7 male rats from the control female rats and the other 28 male rats from female rats that received IP VPA during pregnancy for other groups as follows untreated VPA-induced autism, Risperidone-treated group (at a dose of 2.5 mg /kg IP from postnatal day 23 to 43), prenatal Silymarin-treated group and postnatal Silymarin-treated group in a dose of 200mg/kg orally for 15 days. Neurobehavioral assessment was conducted using the open field and elevated plus-maze tests. Oxidative stress markers in the prefrontal cortex (PFC) were measured with histopathological analysis of the hippocampus and cerebellum. **Results:** VPA exposure impaired neurobehavioral performance, increased MDA values, reduced GSH levels in the PFC and increased the dystrophic changes in the hippocampus and cerebellum. Risperidone and Silymarin improved neurobehavioral performance, reduced oxidative stress markers and attenuated the degeneration in the hippocampus and cerebellum. **Conclusions:** So, we can conclude that Silymarin as therapeutic and prophylactic treatment might improve the autistic-like behavior in VPA-induced autism through its antioxidant effect.

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Introduction

Autism is a NDD known by two crucial manifestations; impairment of social interactions and repetitive behaviors ***(1).*** In addition, these crucial symptoms may be associated with anxiety, irritability, sensory and sleep disorders ***(2)***. According to ***(3)*** Children with ASD represent about one percent, and it is particularly common among males. The pathophysiology of ASD is still unclear, but it was greatly accepted that ASD may result from a mixture of genetic and environmental factors ***(4).*** Nowadays, the treatment of ASD is still a mystery as its etiology is unknown ***(5)***. There are many models undertaken to produce autism in rats, but prenatal exposure to Valproic acid (VPA), a gamma-aminobutyric acid (GABA) agonist anti-epileptic medication, is the most common rodent model of ASD ***(6)***. It has been shown that both ASD cases and the rodents subjected to VPA exhibit many pathological alterations in the hippocampus comprising pyramidal cell dystrophy, increased oxidative stress, apoptosis and neuroinflammation ***(7)***. Moreover, there are dystrophic changes and decreased number of the Purkinje neurons in the cerebellum ***(8).*** Some clinical reports show that activated glial cell has a role in pathophysiology of autism ***(9).*** There is also decrease in expression of BDNF mRNA regarding animals exposed to VPA ***(10).*** Recently, growing research demonstrates that herbal compounds and alternative medicine induce a novel avenue in the context of disease management.

Silymarin, a flavonoid of plant origin derived from Silybum marianum, has been considered a potential agent utilized in the management of hepatic disorders, including alcoholic liver disease and viral hepatitis ***(11).*** In addition to its hepatoprotective effects, Silymarin has recently been considered as neuroprotective drug against many neurodegenerative disorders comprising Alzheimer's disease (AD) ***(12),*** Parkinson's disease (PD) ***(13)***, and cerebral ischemia ***(14). (15)*** reported that it has the ability to inhibit oxidative stress together with its role in many inflammatory and apoptotic pathways. So, on the basis of recent data about silymarin, we hypothesized that it may have neuroprotective effects against the pathophysiologic changes observed in autistic disorders.

Materials and Methods

Experimental Protocol:

35 Sprague-Dawley male rats with a mean weight of 115-200 g were used in this study. Rats were purchased and housed in the animal house of medical experimental research center (MERC), Faculty of medicine, Mansoura University. They were provided with normal chow and water ad libitum at 25 ± 2 ^{o}C with inverted dark-light cycle. All experimental approaches were approved by our local committee of animal care and ethics (Code # MU-ACUC (MED.MS.23.03.8).

Rats were divided into 5 groups;

a) **Control group:** included male offspring whose mothers received intraperitoneal (IP) saline at 12.5 day of gestation.

b) **VPA group:** included male offspring whose mothers received single IP injection of VPA at a dose of 500 mg/kg B.W. at 12.5 day of gestation ***(16).***

c) **VPA+Risp group:** included male offspring from valproic acid treated mothers. They received Risperidone at a dose of 2.5 mg /kg

B.W. (one and half 2 mg Risperdal were crushed and dissolved in 12 ml saline) IP from postnatal day 23 to 43) ***(17).***

d) **VPA+Sylm-Therap group**: included male offspring from VPA-treated mothers. They received silymarin postnatally in a dose of 200mg/kg B.W. orally (prepared 400 mg/10 ml saline) for 15 days ***(15).***

e) **Sylm+VPA-Prophyl group:** included male offspring whose mothers received single IP injection of VPA at a dose of 500 mg/kg B.W. at 12.5 day of gestation plus Silymarin in a dose of 200 mg /kg orally for 15 days.

Drugs and chemicals:

a) **Valproic acid (VPA)** (2-propylpentanoic acid, sodium salt, 98% white pure powder) purchased from Across Organics, USA dissolved to 200 mg/ml in saline.

b) **Risperidone** (Risperdal 2 mg/tablet) purchased from Janssen-Cilag Pharmaceutica; Belgium prepared as 3mg in 12 ml saline.

c) **Silymarin** (milk thistle extract) purchased from Now Family, USA prepared as 400mg in 10 ml saline.

Neurobehavioral assessment:

a) Anxiety-like behaviour by elevated plus-maze test (PND 45):

Wood maze consisting of two open arms (50 cm ×10 cm) opposite to each other and two closed arms (50 cm × 10 cm) opposite to each other and closed by 40cm walls. The maze was elevated 50 cm from the floor to trigger anxiety in rats ***(17).*** Each rat was positioned in the center of the maze facing an open arm and was permitted to move freely for five minutes **(*18*).** The total duration in the open arm and closed arm was recorded. In addition, the number of entries in both arms were measured ***(17).***

b) General locomotive activity and exploratory level using Open field test (PND 48): This test was conducted in a box with transparent walls and its floor is divided into sixteen minor squares**.** Firstly, the animal was positioned in the center box and moved freely within the box for five minutes. Then, motor performance comprising the traveled distance (in cm) and the time spent (in seconds) in each of the central and peripheral regions of the box was reported by a camera ***(19).*** Also, rearing and grooming frequencies are recorded.

Animal sacrifice and brain harvesting:

At the termination of the behavioural testing, rats were anesthetized and sacrificed using intraperitoneal Na-thiopental (at high dose 75 mg/Kg B.W.) ***(20).*** For biochemical studies and analyses, the brains in half of rats in each group were perfused with 150 ml saline for removal of blood clots via cardiac catheterization, then stored at – 80 ^{o}C for future studies and examinations. In contrast, for histopathological examinations, the brains of the reminder rats were collected perfused by 50 ml saline followed by 150 ml formalin via cardiac catheterization, then the brain were stored in 10% formaldehyde.

Biochemical assessment for oxidative stress markers, GSH and MDA:

Prefrontal cortices of rats were homogenized in 5-10 mL cold phosphate-buffered saline (50 mM) in EDTA (one mM) at pH 7.5, after that centrifuged at 4000 rpm for fifteen min at 4°C. Colorimetric assay of the markers was conducted by utilizing commercially available kits (Bio Diagnostics,

Giza, Egypt) based on the manufacturer's instructions.

Histopathological examination:

Histopathological evaluation was performed under light microscope for evaluation of hippocampal damage according to neuronal dystrophy in the CA1 and CA3 areas. At least two coronal sections from different hippocampal levels were examined for each animal. To calculate the neuronal damage percentage, the number of normal neurons was subtracted from the total neuron count in the hippocampal areas (CA1 and CA3) per coronal section, and this result was divided by the total cell count. The results were graded as follows: 0 (no change), I (mild change: 1–25% neuronal degeneration), II (moderate change: 26–75% neuronal degeneration), or III (severe changes: 76–100% neuronal degeneration) ***(21).*** Also, the average number of dystrophic Purkinje cells (PCs) in the cerebellum is calculated.

Statistical analysis:

Results were statistically analyzed by using SPSS (SPSS 25.0, IBM/SPSS Inc., and Chicago, IL). One-way ANOVA test with Tukey's test was utilized to determine the statistical significance differences in our assessment. Data were considered significant when $p \leq 0.05$.

Results

Results of General locomotive activity and exploratory level using Open field test (PND 48):

The results of rats' spontaneous behavior in open field test are demonstrated in Figure (1): VPA group displayed non-significant difference compared with the controls and VPA+Risp group displayed significant reduction in travelled distance compared to VPA group (p=0.021), but compared to VPA+Risp, there was a significant increase in VPA+Sylm-Therap (P=0.017) and Sylm+VPA-Prophyl group (P=0.009). Non-significant difference was noticed between VPA+Sylm-Therap and Sylm+VPA-Prophyl groups. VPA group displayed significant reduction in time spent in the centre compared with the controls (P=0.015). Moreover, all treated groups (VPA+Risp, VPA+Sylm-Therap and Sylm+VPA-Prophyl) displayed significant increase compared to VPA group and VPA+Sylm-Therap group displayed significant reduction compared to VPA+Risp group (P=0.004). No significant difference between VPA+Sylm-Therap and Sylm+VPA-Prophyl groups. Regarding the time spent in the periphery, there wasn't any significant difference among study groups. VPA group displayed significant increase in the number of grooming compared with the controls (P=0.004) and significant reduction in VPA+Risp and VPA+Sylm-Therap groups compared to VPA group (P=0.002 and 0.011 correspondingly). Also there was insignificant difference between VPA+Sylm-Therap and Sylm+VPA-Prophyl groups. The number of rearing displayed significant increase in VPA group and Sylm+VPA-Prophyl group compared with the controls (P=0.01 and 0.05 correspondingly). Moreover, there was significant increase in Sylm+VPA-Prophyl group compared to VPA+Risp group (P=0.04). No significant difference between VPA+Sylm-Therap and Sylm+VPA-Prophyl groups.

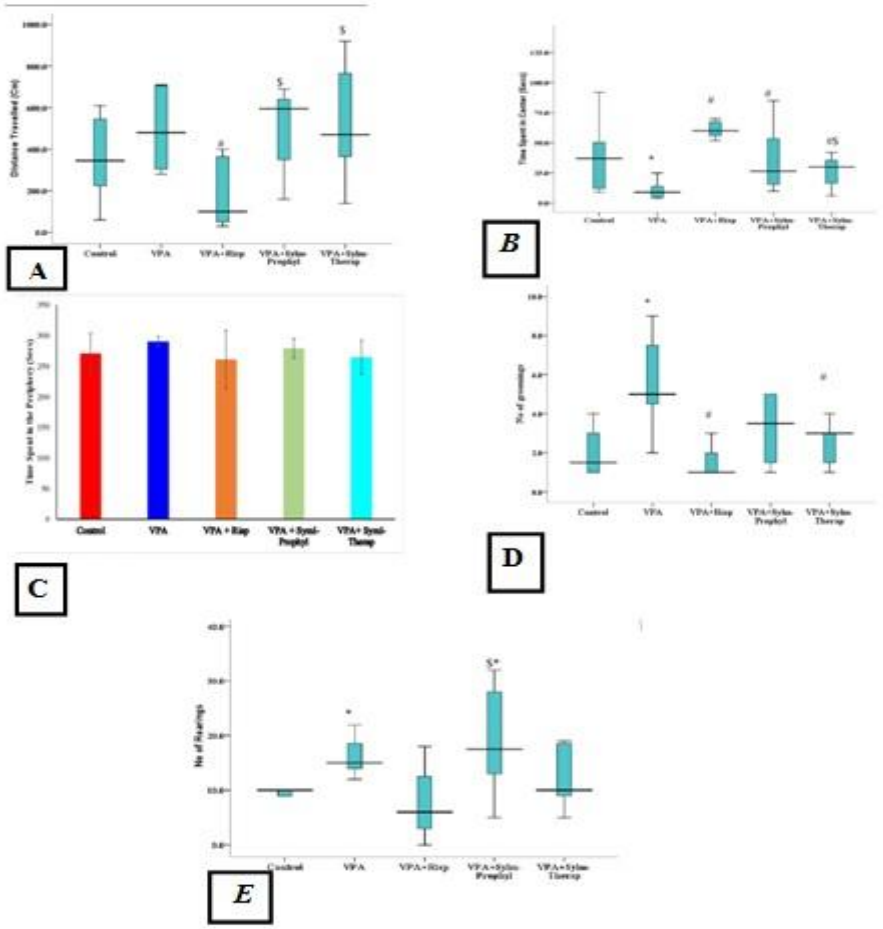

Figure (1) Effect of VPA, Risperidone and Silymarin on rats' spontaneous behavior in open field test. (A) The distance travelled (cm), (B) the time spent in the centre, (C) The time spent in the periphery, (D) the number of grooming and (E) The number of rearing. Data are expressed as median for non-parametric data and mean± SD for parametric data. KW: Kruskal Wallis test. F for ANOVA test. * Significant by Mann-Whitney. * Significant versus control group, # significant versus VPA group and $ significant versus VPA + Risp group. P ≤ 0.05 is considered significant.

Anxiety-like behavior by elevated plus-maze test (PND 45):

The results of Anxiety-like behavior by elevated plus-maze test are demonstrated in Fig. (2). VPA displayed non-significant difference with control group and also all treated groups displayed non-significant difference with VPA group and with each other except Sylm+VPA-Prophyl group displayed significant increase in number of entries in open arms compared to VPA group (P=0.001). The number of entries in closed arms did not display significant difference among the study groups. Regarding time spent in open arms, VPA group displayed significant reduction compared with the controls (P=0.004). Moreover, all treated groups (VPA+Risp, VPA+Sylm-Therap and Sylm+VPA-Prophyl) displayed significant increase compared to VPA group. No significant difference between VPA+Risp and VPA+Sylm-Therap or Sylm+VPA-Prophyl groups and also non-significant difference between VPA+Sylm-Therap and Sylm+VPA-Prophyl groups. All treated groups (VPA+Risp, VPA+Sylm-Therap and Sylm+VPA-Prophyl) displayed a significant decrease in time spent in closed arms compared to VPA group. No significant difference between VPA+Risp and VPA+Sylm-Therap or Sylm+VPA-Prophyl groups and also non-significant difference between VPA+Sylm-Therap and Sylm+VPA-Prophyl groups.

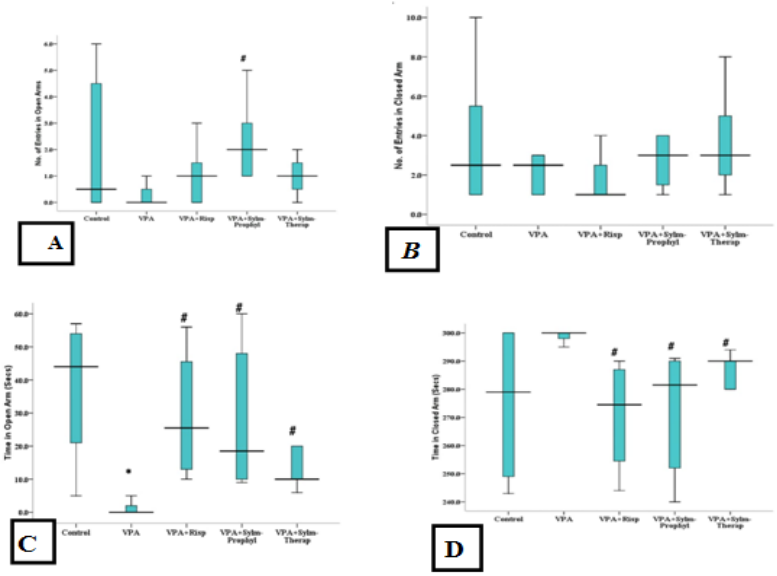

Fig. (2) Effect of VPA, Risperidone and Silymarin on Anxiety-like behavior by elevated plus-maze test. (A) The number of entries in open arms, (B) The number of entries in closed arms, (C) The time spent in open arms and (D) The time spent in closed arms. Data are expressed as median (minimum- maximum), KW: Kruskal Wallis test. * Significant by Mann-Whitney. * Significant versus control group, # significant versus VPA group and $ significant versus VPA + Risp group. P ≤ 0.05 is considered significant.

Biochemical assessment for oxidative stress markers, GSH and MDA:

Fig. (3A) displays the concentration of MDA in PFC in various groups. VPA displayed a significant increase compared with the controls **(P=0.002),** however other groups did not display significant difference compared to VPA group and among each other. ***Figure (3B)*** displays the concentration of GSH in PFC in various groups. Compared with the controls, there was significant reduction in GSH concentration in all studied groups (VPA, VPA+Risp, VPA+Sylm-Therap and Sylm+VPA-Prophyl). In contrast, VPA+Sylm-Therap group displayed significant increase compared with VPA **(p< 0.001)** and VPA+Risp group **(P=0.002)**. No significant difference between VPA+Sylm-Therap and Sylm+VPA-Prophyl groups.

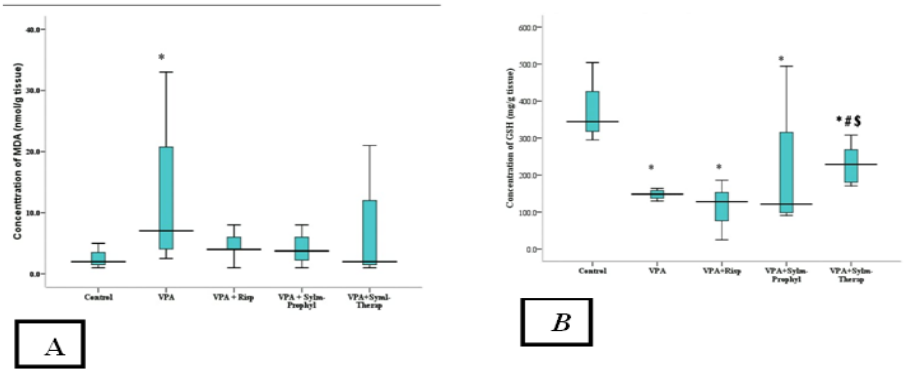

Fig. (3) Effect of VPA, Risperidone and Silymarin on MDA and GSH levels. (A) The concentration of MDA in PFC of different studied groups and (B) The concentration of GSH in PFC in different studied groups. Data are expressed as median (minimum- maximum), KW: Kruskal Wallis test. * Significant by Mann-Whitney. * Significant by Mann-Whitney. * Significant versus control group, # significant versus VPA group and $ significant versus VPA + Risp group. P ≤ 0.05 is considered significant.

Results of histopathological examination of the hippocampus and cerebellum using Hematoxylin and Eosin:

a) Hippocampal CA1 histopathological examination

(Fig 4A) shows the score of neuronal dystrophy in CA1 region of hippocampus among studied groups. All studied groups displayed significant increase in dystrophic neurons compared with the controls**.** Compared to VPA group, there was significant decrease in this score in VPA+Risp group. Moreover, it was significantly elevated in Sylm+VPA-Prophyl group compared with VPA+Risp group, but non-significant difference between VPA+Risp and VPA+ Sylm-Therap groups .Also non-significant difference was detected between VPA+Sylm-Therap and Sylm+VPA-Prophyl groups.

Regarding histopathological findings, the control group displayed a typical neuronal structure with round nuclei and prominent nucleoli and less number of dystrophic neurons indicated by the red arrow. The VPA group displayed low number of round normal pyramidal cells and increased number of dystrophic neurons which are shrunken and have condensed chromatin. VPA+Risp and VPA+Sylm-Therap groups displayed increase in the number of pyramidal cells with some restoration of the normal shape of pyramidal cells. Sylm+VPA-Prophyl group displayed increase in the number of pyramidal cells with less evidence of neuronal morphologic improvement concerning nuclear and cytoplasmic features (***Fig 4B-F)*** correspondingly.

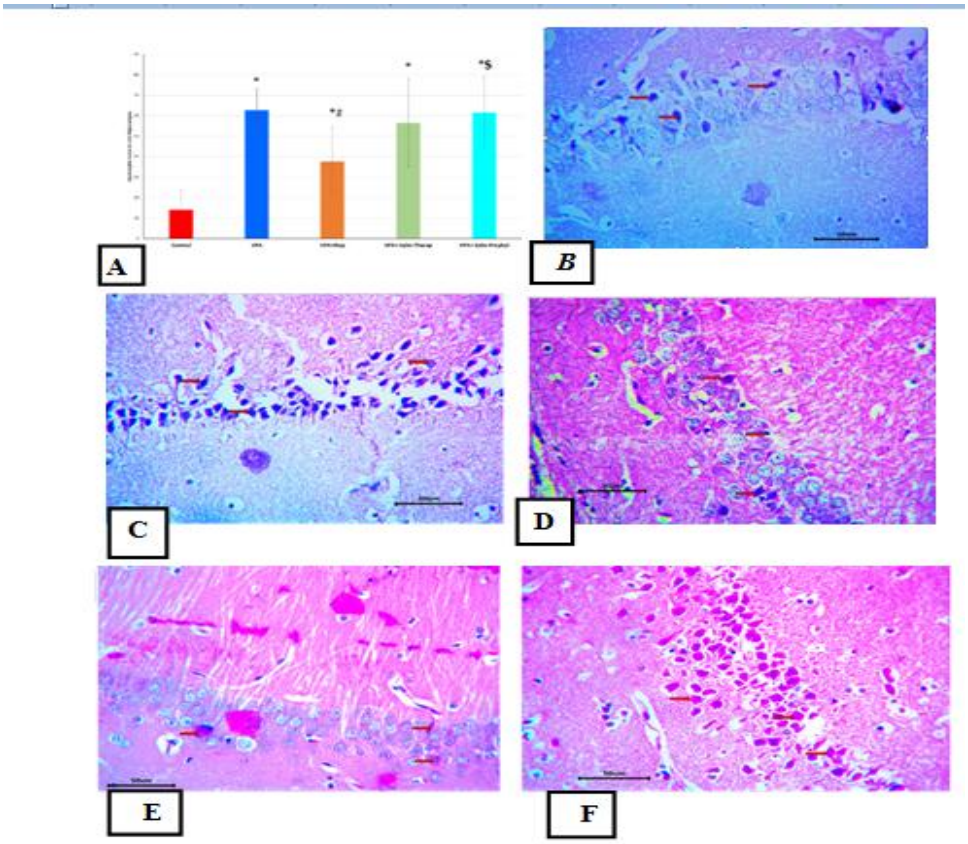

Fig. (4): Effect of VPA, Risperidone and Silymarin on the CA1 region of hippocampus. (A) Shows the score of neuronal dystrophies in CA1 region of hippocampus among different groups. Data are mean± SD. * significant versus control group, # significant versus VPA group and $ significant versus VPA+Risp group. Control (B), VPA group (C), VPA + Risp (D), VPA+ Sylm-Therap (E) and VPA+ Sylm-Prophyl (F). Red arrows indicate the dysmorphic neurons. The scale bars represent 50 µm at magnification 400x, (H&E).

b) Hippocampal CA3 histopathological examination:

Figure (5A) shows the score of neuronal dystrophy in CA3 region of hippocampus among studied groups. VPA group and Sylm+VPA-Prophyl group displayed significant increase in dystrophic neurons compared with the controls. In contrast, VPA+Risp group only displayed significant reduction compared to VPA group. Moreover, Sylm+VPA-Prophyl group displayed significant increase compared with VPA+Risp group with non-significant difference was noticed with VPA+Sylm-Therap group. Also there was non-significant difference between VPA+Sylm-Therap and Sylm+VPA-Prophyl groups.

H&E stain in control group displayed Pyramidal cells with round vesicular nuclei, prominent cytoplasmic processes and normal distribution of cells. In contrast, VPA group displayed sparse arrangement of pyramidal cells with increased intercellular gaps and reduction in cell number. VPA group also shows shrunken cells with condensed chromatin. VPA+Risp and VPA+Sylm-Therap groups displayed increase in the number of normal pyramidal cells regarding the nuclear and cytoplasmic features. However, Sylm+VPA-Prophyl group displayed marked elevation in the number of dystrophic neurons even more than VPA group ***(Fig 5B-F)*** correspondingly.

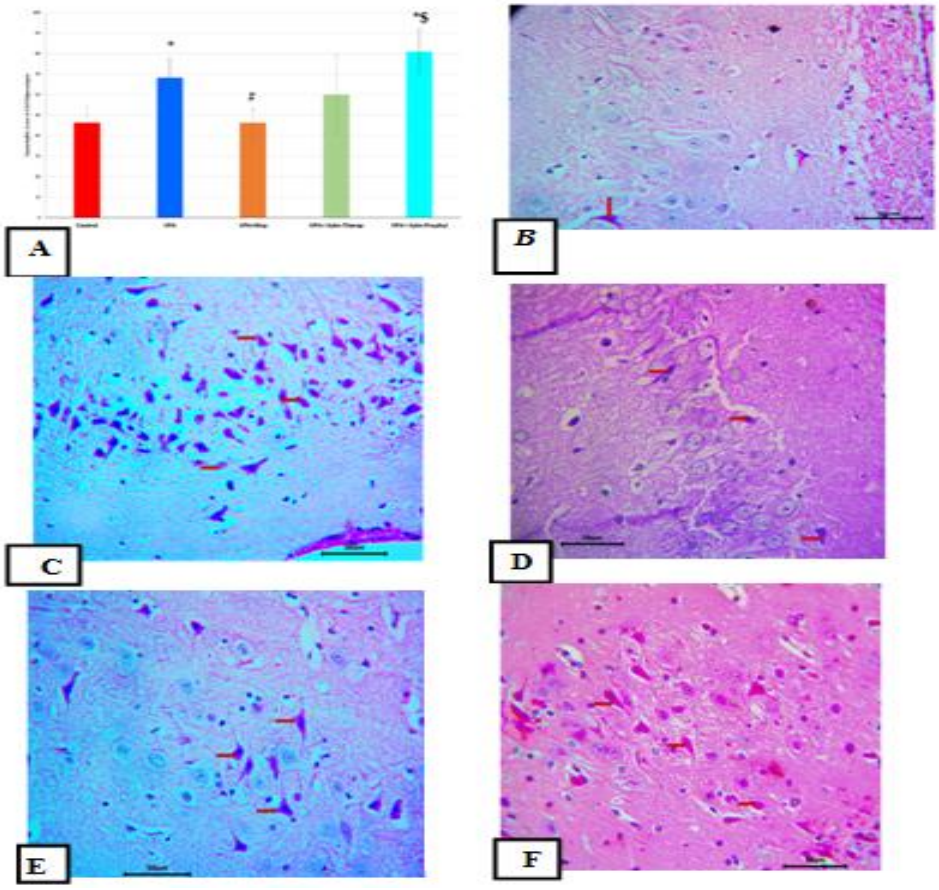

Fig. (5): Effect of VPA, Risperidone and Silymarin on the CA3 region of hippocampus. (A) Shows the score of neuronal dystrophies in CA3 region of hippocampus among different groups. Data are mean± SD. * significant versus control group, # significant versus VPA group and $ significant versus VPA+Risp group. Control (B), VPA group (C), VPA + Risp (D), VPA+ Sylm-Therap (E) and VPA+ Sylm-Prophyl (F). Red arrows indicate the dysmorphic neurons. The scale bars represent 50 µm at magnification 400x, (H&E).

c) Cerebellum histopathological examination :

Figure (6A) displays the number of dystrophic PCs in the cerebellum among studied groups**.** VPA group displayed significant increase in the number of dystrophic PCs compared with the controls. In contrast, VPA+Risp group, VPA+Sylm-Therap group and Sylm+VPA-Prophyl group displayed significant reduction compared to VPA group. The histopathological findings in the control group revealed that the cerebellar cortex is composed of three layers; outer molecular layer (ML), middle Purkinje cell layer (PC), and inner granular cell layer (GL). Moreover the PCs show round vesicular nuclei and prominent cytoplasmic processes. In contrast, VPA group shows decrease in the number of PCs which are shrunken and disfigured with condensed chromatin. VPA+Risp and VPA+Sylm-Therap groups show normal shape of cerebellar cortex and increased number and thickness of PCs compared to VPA group. Sylm+VPA-Prophyl group shows normal shape of cerebellar cortex and more increase in Purkinje cell number and thickness compared to VPA group, but less than VPA+Risp group ***(Fig 6B-F)*** correspondingly.

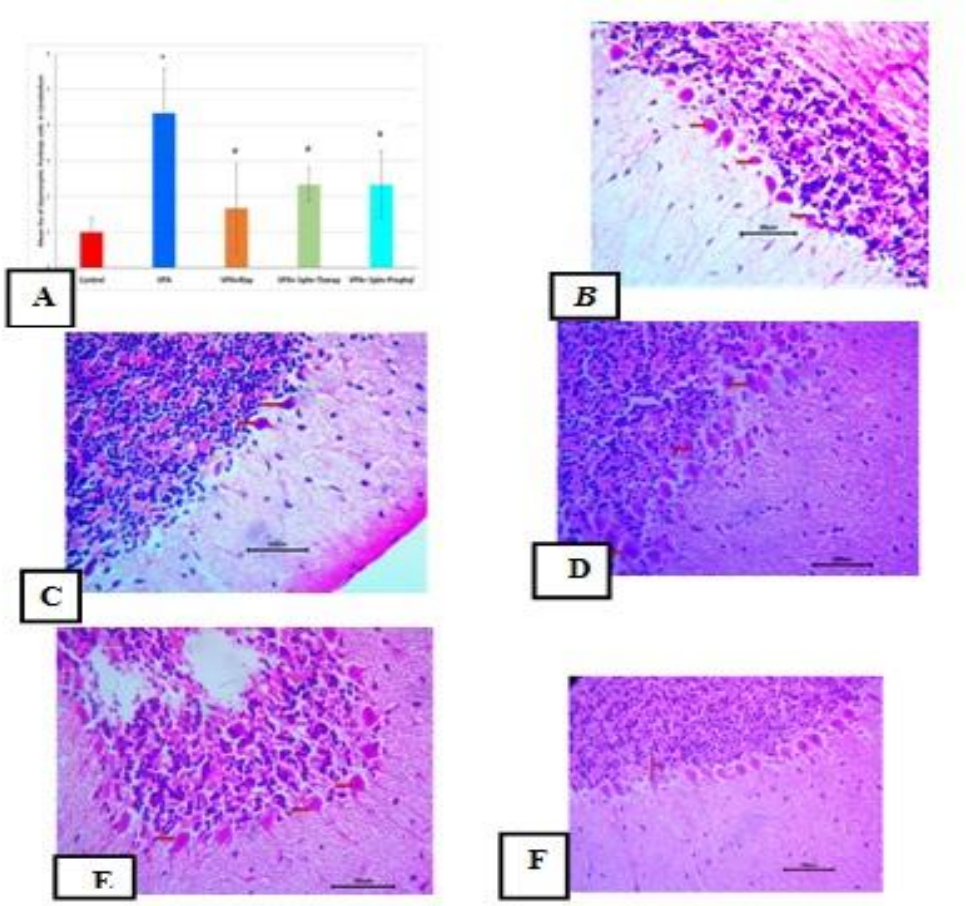

Fig. (6): Effect of VPA, Risperidone and Silymarin on the cerebellum. (A) The number of dystrophic PCs in Cerebellum among different groups. Data are mean± SD. * significant versus control group, # significant versus VPA group and $ significant versus VPA+Risp group. Control (B), VPA group (C), VPA + Risp (D), VPA+ Sylm-Therap (E) and VPA+ Sylm-Prophyl (F). Red arrows indicate the dysmorphic neurons. The scale bars represent 50 µm at magnification 400x, (H&E).

Discussion

Autism has been considered a NDD characterized by impairment of social communication and repetitive behaviors **(22)**. The prevalence of ASD is rising all over the world, and the average age to diagnose ASD has decreased **(23).** The hippocampus has been a key focus in ASD research due to its role in memory, learning, language, emotions and cognition **(24).** Additionally, the cerebellum has a role in cognitive and affective processes, comprising attention, language ability, working memory and emotions. So, it is suspected that cerebellar changes may participate in the different symptoms noticed in subjects with autism **(25).** One promising therapeutic compound is Silymarin that is mainly used against different liver diseases. It was proven to exhibit antioxidant properties and much research has recorded Silymarin's neuroprotective mechanisms against different types of neurologic conditions **(26).** The current study was undertaken to evaluate the effect of Silymarin on cerebellar cortex and hippocampus morphology, behavioral changes and oxidative stress in rat model of VPA-induced autism.

In the present study, the neurobehavioral tests were carried out on the rats at days PND 45 and PND 48, one dose of VPA (500mg/kg on gestational day 12.5th) significantly produced changes in the rat behavior and motor performance. This was reported previously by **(16, 27). (28)** reported that they found autistic –like behaviors in rat offspring when their mothers exposed to VPA (400mg/kg) on gestational day 12.5. In addition, these results confirmed the development of autism after VPA 500 mg/Kg. Regarding the open field test in VPA group, there was increase in travelled distance in VPA-treated group compared with the remaining groups. **(29)** confirmed our results and proved the increased activity of the VPA-exposed rats. Regarding time spent in the centre, our results revealed significant reduction in VPA group compared to the remaining groups, while time spent in the periphery revealed increase in VPA group compared with other groups. This is in accordance with **(30)** who displayed that VPA rats spent less time in the central zone during the open field test, which indicates anxiety in animal models. Moreover, grooming frequency was significantly increased in VPA-treated group compared with the controls. Similarly, **(31)** reported that VPA mice displayed increased repetitive self-grooming. Regarding rearing frequency, VPA group displayed significant increase compared with the controls. **(32)** previously reported that there was a considerable increase noticed in the number of rearing in the VPA-treated animals compared with the controls. In contrast, **(33)** disagreed with our results and reported that induction of autism significantly decreased rearing because he considered rearing as exploratory behavior.

Open field test in VPA group treated with Risperidone revealed significant reduction in the travelled distance with significant increase in the time spent in the centre compared to VPA group. In addition, it displayed non-significant reduction in the time spent in the periphery and rate of rearing compared to VPA group. Moreover, it displayed significant reduction in frequency of grooming compared to VPA group. These results

were in agreement with (**34)** who demonstrated that Risperidone could improve social interaction. Therapeutic and prophylactic groups of Silymarin displayed significant increase in travelled distance compared to VPA group treated with Risperidone. Moreover, both groups displayed significant increase in the time spent in the centre compared to VPA group. Concerning time spent in the periphery, there wasn't any significant difference between both groups and other groups. Therapeutic group of Silymarin displayed significant reduction in grooming frequency, while prophylactic group of Silymarin displayed non-significant reduction compared to VPA group. Regarding rearing frequency, prophylactic group of Silymarin displayed significant increase, but therapeutic group of Silymarin displayed non-significant increase compared to Risperidone-treated group. This support the role of Silymarin in improvement of locomotor activity and exploratory behavior in rats and this was in agreement with **(35).**

To test anxiety-like behaviour, elevated plus-maze test was done. Our study found that the number of entries and the time spent in open arms were diminished in VPA group compared to other groups. Our results were in alignment with those reported by **(36)** and indicate anxiety-like behavior. The number of entries in closed arms displayed non- significant reduction in VPA group compared to the controls. **(37)** confirmed our results and displayed that prenatal exposure to VPA decreased the number of entries in the opened arms and also in the closed arms. **(17)** disagreed with our results and reported an increased number of entries in closed arms in VPA-induced autism. Time spent in closed arms displayed increase in VPA group compared to other groups and this in accordance with **(38)**. Risperidone-treated group did not display significant difference compared to other groups regarding the number of entries in open arms and number of entries in closed arms. However, compared to the VPA group, it exhibited a notable increase in the duration spent in open arms and a significant decrease in the time spent in closed arms. This was previously reported by **(39)**. Therapeutic group of Silymarin did not display significant difference in number of entries in open and closed arms in elevated plus-maze test compared to other groups, however prophylactic group of Silymarin displayed significant increase in number of entries in open arms and non-significant increase in number of entries in closed arms compared to VPA group. Both groups displayed significant elevation in time spent in open arms and significant reduction in time spent in closed arms compared with VPA group. This suggested a preventive role of Silymarin in anxiety. So, Silymarin as therapeutic and prophylactic treatment improve autistic-like behaviors and anxiety in the offspring of mothers treated with VPA, denoting that Silymarin may have a neuroprotective effect in rat model of autism. In accordance with our findings, several studies demonstrated the useful actions of Silymarin on neurologic disorders such as neurodegenerative diseases including AD, PD, and cerebral ischemia. This was confirmed by **(12, 15, and 40)**

It has been recommended that oxidative stress might be an important characteristic in ASD **(41)**. In our study, we found a significant elevation in oxidative stress marker (MDA) and decrease in the

antioxidant **(**diminished glutathione (GSH)**)** in VPA group compared with the controls. This was previously confirmed by **(42)**. Risperidone-treated group displayed non-significant reduction in MDA and GSH levels compared to VPA group. This was disagreed with **(43).** However, Silymarin as therapeutic treatment displayed non-significant reduction in MDA level and significant increase in GSH level compared to Risperidone-treated group and VPA group. Prophylactic group of Silymarin displayed non-significant reduction in MDA and GSH levels compared to Risperidone-treated group. In accordance with our results, Silymarin when administered at a dosage of 200 mg/kg/day, greatly diminished the oxidative stress in hippocampus and cortex of elderly rats in comparison to the young ones and this was reported by **(44).**

Evidence of neurodegeneration in the brain in ASD includes: neuronal cell dystrophy, activation of microglia and astrocytes, neuroinflammation and oxidative stress **(45**). Preceding researches revealed a reduction in PCs in the cerebellum, as well as changes in the number and size of other neurons **(46)**. In our study, we found increase in the number of dystrophic PCs in the cerebellum of VPA group compared with other groups. Risperidone-treated group demonstrated significant decrease in the number of dystrophic PCs compared to VPA group and this was reported previously by **(47)**. Also, Silymarin as therapeutic and prophylactic treatment displayed significant reduction in the number of dystrophic PCs. This was previously reported by **(48). (30)** reported that multiple cerebral areas are involved in ASD comprising loss of CA1 and CA3 pyramidal neurons in the hippocampus. In our study, we found that increase in the score of neuronal dystrophy in CA1 and CA3 areas of the hippocampus in VPA group compared to other groups. Risperidone-treated group displayed decrease in the score of neuronal dystrophy in CA1 and CA3 areas of hippocampus compared to VPA group and this was in agreement with **(49).** Therapeutic group of Silymarin displayed reduction in the score of neuronal dystrophy in CA1 and CA3 areas of hippocampus compared to VPA group while prophylactic group of Silymarin displayed slight decrease in neuronal dystrophy in CA1 region of hippocampus and increase in neuronal dystrophy in CA3 region of hippocampus compared with VPA group.

Further researches are needed to explain some regional differences in some parameters like Silymarin as prophylactic treatment decrease neuronal dystrophy in CA1 region of hippocampus, while dystrophic neurons were increased in CA3 region of the hippocampus compared to VPA group.

Conclusions

Therapeutic and prophylactic Silymarin treatments exhibit potential neuroprotective effects in a rat model of VPA-induced autism. Silymarin improves neurobehavioral performance, reduces neuronal degeneration, and mitigates oxidative stress by increasing GSH values and reducing MDA values. We conclude that Risperidone-treated group and Silymarin-treated groups displayed improvement in neurobehavioral, biochemical and histopathological assessment, but Risperidone-treated group displayed better results in neurobehavioral assessment. So, Silymarin

could therefore be a promising therapy for the management of autistic disorder but much research is necessitated to explain Silymarin's mechanisms in neuroprotection.

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