Original Research Article

The Possible Effect of Celastrol on Ameliorating Mitochondrial Dysfunction and Neuro-inflammation in Sodium Valproate Induced- Rat Model of Autism

AmlAlaa El-Din El-Sayed Ahmed[1], Samia HussienAbou El-Seoud[2], Fleur Fathi Abdel Moneim[2], Rasha Osama El-Esawy[3]

[1]Demonstrator of Pharmacology, [2]Professor of Pharmacology, [3]Lecturer of Pharmacology, Faculty of Medicine, Tanta University, Egypt

Abstract

Autism spectrum disorder (ASD) is a neurodevelopmental disease with impairment in social interactions, language and repetitive stereotypical behaviors. Celastrol is a natural safe compound that has anti-inflammatory and as a neuroprotective effects. 48 male offspringswistar rats divided into 6 groups; normal control group, offsprings receive vehicle, autistic offsprings receive vehicle, autistic offsprings receive risperidone, autistic offspringsreceive celastrol, autistic offspringsreceive both risperidone& celastrol. At the end of experiment behavioral tests were performed then neurochemical analysis and histopathological examination. The obtained data showed that celastrol improved social deficits, decreased repetitive/restricted behaviors in T-maze test, significant increase in SIRT-1, GSH level with significant decrease in DRP-1, IL-6, caspase-3 and MDA with amelioration of histopathological findings in VPA-induced ASD in both cerebellum and hippocampus. These findings pave the way for using celastrol as an adjuvant therapy during long-term clinical use of risperidone in ASD**.**

Keywords: *ASD, celastrol and mitochondrial dysfunction.*

Introduction

Autism spectrum disorder (ASD) is a neuro-developmental disorder that affect 1.1% of children in first 3 years of life with male: female ratio of 5:1**(1).** It is characterized by impairments in social interaction, deficits in verbal and nonverbal communication along with stereotyped and repetitive behaviors**(2).**

The exact aetiology of ASD is unknown although many hypothesis suggest several factors including genetic predisposition, mitochondrial dysfunction, oxidative stress, inflammation and environmental toxicant exposure**(3)**.

Current available pharmacotherapeutic options of autism are only symptomatic with various side effects**(4)**. Although, risperidone and aripiprazole are the only two psychotropic drugs that have been approved by the US Food and Drug Administration (FDA) for the treatment of autistic children**(5)**, However, they fail to improve the core behavioral alterations of autism.

Corresponding author:
Dr. Rasha Osama El-Esawy
Lecturer of Pharmacology, Faculty of Medicine,
Tanta University, El-Geesh St, Tanta, Egypt.
E-mail: rasha.elesawy@med.tanta.edu.eg
Postal code: 31511

Furthermore, considerable limitations are associated with their long term therapy[6]**.** The most common adverse effects occurring with risperidone long term therapy are significant weight gain, somnolence**,** hyperprolactinemia, diabetes mellitus [7]. So, there is an urgent need for development of new and safe disease modifying therapies that target the underlying pathophysiology of the disease with minimal side effects. The aim of this study was to evaluate the possible effect of celastrol alone and in combination with risperidone on amelioration of mitochondrial dysfunction, its anti-inflammatory, antioxidant effects in VPA induced rat model of ASD.

Celastrol is a natural pentacyclic triterpenoid derived from the root extracts of Tripterygium wilfordii of the Celastraceae family[8]. The therapeutic potential of this compound came from its safety [9] and efficacy as anti-inflammatory[10], anti-oxidant, neuroprotective and amelioration of mitochondrial dysfunction[11].

Materials and Methods

Drugs and chemicals

Celastrol dissolved in DMSO, administrated orally by oral gavage in a dose of 20mg/kg/day[12], Risperidone dissolved in DMSO, administrated orally by oral gavage in a dose of 2mg/kg/day[13], both obtained from (AdooQBioScience, Califronua, USA), valproic acid sodium salt dissolved in saline at concentration of 250mg/ml was administrated by intraperitoneal injection (i.p.) in a dose of 400mg/kg[14](Sigma, St., Louis, MO, USA).

Animals and study design

60 female wistar rats were mated overnight, two females were allowed to mate with one male in the same cage, in the morning when vaginal plug was found it is defined as the first day of gestation[15]**.** Females injected by 400mg/kg sodium valproate single intraperitoneal injection on the 12.5 day of gestation [16]. Each female was individually housed to allow her to put her own litters, after weaning male offsprings were divided into 6 equal groups (**Fig.1**). Group 1: Normal control group, Group 2: Offsprings of female wistar rats (treated with normal saline) received vehicle from post-natal day (PND) 21st -35th, Group 3: Autistic offsprings of female wistar rats (treated by VPA) received vehicle from PND 21st -35th, Group 4: Autistic offsprings of female wistar rats (treated by VPA) received risperidone from PND21st -35th, Group 5: Autistic offsprings of female wistar rats (treated by VPA) received celastrol from PND21st -35th, Group 6: Autistic offsprings of female wistar rats (treated by VPA) received both risperidone&celastrol from PND 21st -35th. At the end of experiment behavioral tests including three chamber test and T-maze were performed.

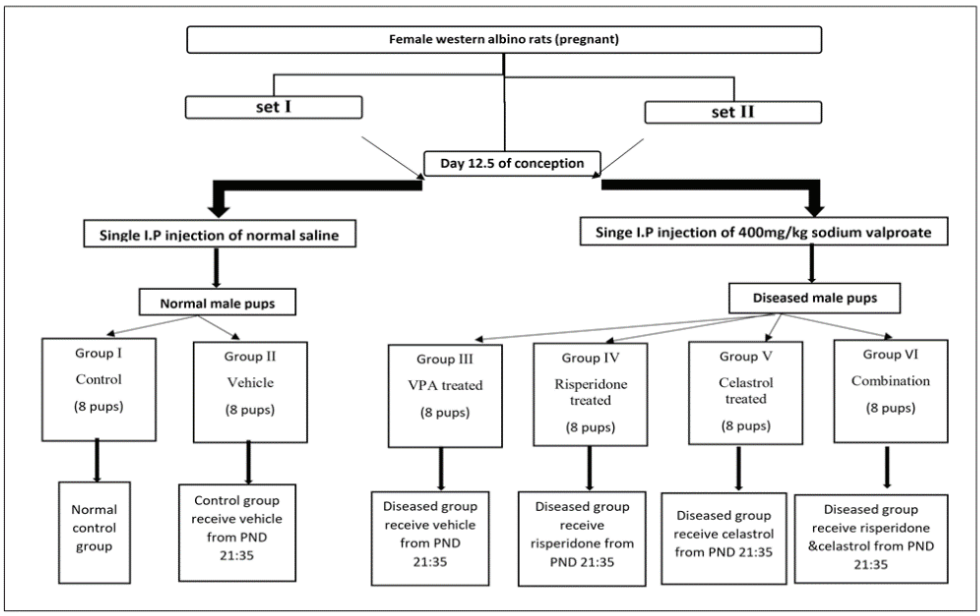

Fig. (1): Experimental design of the study.

Three chamber test is for social interaction and social novelty interest. Sociability is a significant tendency to spend time with stranger rat rather than spending time in empty chamber, while social noveltyinterest is a significant tendency to spend time with a new rat rather than familiar one.Apparatus is a rectangular box divided by clear Plexiglas walls into equal three chamber, each one is 19 x 45 cm. The dividing walls have an open middle section to allow free movements in between three chambers.

Two large identical wire cups that can hold a single rat were used. Test was started by habituation, subjected rat was placed in the center of middle chamber for five minutes and opening in the dividing walls were closed by plastic box during habituation, After habituation (session I) was started as stranger 1 was placed in one of the side chambers and enclosed in the wire cup which allow contact and prevent fighting between subjected rat and stranger, subjected rat was allowed to explore whole chambers for 10 minutes, length of time in empty chambers and time spent with stranger 1 were recorded. At the end of this session rat was tested for a second 10 minutes (session II) that test social memory and novelty, stranger 2 was placed inside an identical wire cup in the opposite side chamber that was empty during session I, length of time spent with stranger 1 as well as with stranger 2 were recorded [17].

T-maze testis used to evaluate repetitive/ restricted behavior.Normal rats tend to alternate between arms by their memory, this reflecting their motivation for environmental exploration.For each rat five sessions were performed and the first choice was evaluated[18].T maze is a wooden enclosed apparatus in the form of T placed horizontally with the start alley measuring 30 cm length and 10 cm width, the goal arm also measuring 30 cm length and 10 cm width and wall height is 20 cm[19].The rat was placed for 10 minutes in the examination room, then we put it in the start zone and allowed to choose the goal arm. The rat was confined in the chosen arm for 30 seconds then removed and placed in the home cage for 60

seconds. Afterward, it was taken again to the start arm to begin the 2nd trial. This trial was repeated for 5 consecutive times with 1 minute in between and 30 seconds of habituation in the chosen arm. Percentage of alternation (%) between the left and right arms was analyzed**.**

Biochemical assays

Tissue preparation

The rats were anesthetized by ether and were sacrificed and cerebellum and hippocampus were dissected, washed with phosphate buffered saline (PBS) solution, pH 7.4.to remove any red blood cells and clots. The right half of both cerebellum and hippocampus were fixed in 10 % formalin and processed for examination of histopathological changes by light microscope, while the left half of both cerebellum and hippocampus were stored at (-80ºC) until prepared for the assessment of the tissue parameters.

Determination of mitochondrial parameters (SIRT-1&DRP-1):

Isolation of Mitochondria: We used the left half of both cerebellum and hippocampus for mitochondrial extraction. Tissues were sampled, immediately washed with phosphate-buffered saline, and then homogenized with a glass grinding tube on ice for about 20 times in 1 ml mitochondrial isolation buffer (0.01mol/liter Tris-HCl, 0.0001 mol/liter EDTA-2Na, 0.01mol/liter sucrose, 0.8% NaCl, pH 7.4). The homogenate was kept at (48ºC) and centrifuged at 1,500 rpm for 10 min. The supernatant was collected and then centrifuged again at 10,000 rpm for 15 min. The precipitate is the mitochondria[20]

Determination of tissue sirtuin-1 (SIRT1) (ng/ml):

Rat sirtuin-1 was performed using ELISA kit supplied by biodiagnostic; Catalogue No. 201-11-1498.

Determination of tissue Dynamin related protein 1 (DRP1) (pg/ml):

RatDynamin related protein 1 was performed using ELISA kit supplied by Biodiagnostic; Catalogue No. 201-11-3125.

Determination of tissue caspase-3 (ELISA) (ng/ml):

Rat cysteinyl aspartate specific proteinases 3(Caspase-3/CPP32) was performed using ELISA kit supplied by Sun Red; Catalogue No. 201-11-0281.

Determination of tissue Interleukin-6 (IL-6):

IL-6 was measured in tissue homogenate by kits obtained from Chongqing Biospes Co., Ltd Company, China,catalog No.: BEK1110 according to the method described by[21].

Determination of tissue reduced glutathione (GSH) (mg/ml)

Reduced glutathione (GSH) level assay was performed using Biodiagnostic supplied Kit (Cat. No TA 2511.), based on the Beutler spectrophotometric process, [22]. **Determination of malondialdehyde (MDA) (nmol / ml)**

Lipid peroxidation was assessed by calculating serum malondialdehyde (MDA) levels according to the Ohkawa et al. method [23] using Biodiagnostic supplied kit (Cat. No. MD 2529.

Histopathological examinations

The right half of both cerebellum and hippocampus were fixed in 10 % formalin, stained with hematoxylin and eosin (H&E) and processed for examination of histopathological changes by light microscope.

Statistical Analysis

Data were represented as mean ± standard error

of mean (SEM).The significance was considered at values of P<0.05.

Results

Celastrol improved sociability and social affiliation in VPA-induced ASD:

VPA-treated group (group3) revealed a significant decrease in length of timein minutes spent with stranger 1as compared to the normal control group (group1), indicating impaired sociability. Risperidone treated group (group4) revealed a significant increase in length of timein minutesspent with stranger 1 as compared to the valproate treated group (group3), indicating improved sociability. Celastrol treated group (group5) showed a significant increase in length of timein minutes spent with stranger 1 as compared to the valproate treated group (group3), indicating improved sociability. Combination group (group6) showed a significant increase in length of timein minutesspent with stranger 1 as compared to the valproate treated group (group3), indicating improved sociability, non-significant increase in length of timein minutesspent with stranger 1 as compared to risperidone treated group (group 4), non-significant increase in length of timein minutes spent with stranger 1 as compared to celastrol treated group (group 5).

Celastrol improved social memory &noveltyin VPA-induced ASD:

VPA-treated group (group3) revealed a significant decrease in length of timein minutes spent with stranger 2as compared to the normal control group (group1), indicating decreased social motivation and novelty. Risperidone treated group (group4) revealed a significant increase in length of time in minutesspent with stranger 2 as compared to the valproate treated group (group3), indicating increased social motivation and novelty. Celastrol treated group (group5) showed a significant increase in length of time in minutesspent with stranger 2 as compared to the valproate treated group (group3), indicating increased social motivation and novelty. Combination group (group6) showed a significant increase in length of timein minutesspent with stranger 2 as compared to the valproate treated group (group3), indicating increased social motivation and novelty, non-significant increase in length of timein minutes spent with stranger 2 as compared to risperidone treated group (group 4), non-significant increase in length of time in minutes spent with stranger 2 as compared to celastrol treated group (group 5).

Celastrol improved repetitive/restricted behaviors in VPA-induced ASD :

VPA-treated group (group3) revealed a significant decrease in percentage of alternation as compared to the normal control group (group1), reflecting repetitive/restricted behaviors. Risperidone treated group (group4) revealed a significant increase in percentage of alternation as compared to the valproate treated group (group3), reflecting improvement of repetitive/restricted behaviors. Celastrol treated group (group5) showed a significant increase in percentage of alternation as compared to the valproate treated group (group3), reflecting improvement of repetitive/restricted behaviors. Combination group (group6) showed a significant increase in percentage of alternation as compared to the valproate treated group (group3), reflecting improvement of repetitive/restricted behaviors,significant increase in percentage of alternation as compared to risperidone treated group (group 4), significant increase in percentage of alternation as compared to celastrol treated group (group 5).

Celastrol ameliorated mitochondrial dysfunction in VPA-induced ASD:

VPA-treated group (group3) revealed a significant decrease in SIRT-1 level, significant

increase in DRP-1 level as compared to the normal control group (group1) in both cerebellum, and hippocampus, indicating mitochondrial dysfunction. Risperidone treated group (group4) revealed non-significant difference in SIRT-1 and DRP-1 level as compared to the valproate treated group (group3) in both cerebellum, and hippocampus, indicating no improvement in mitochondrial dysfunction. Celastrol treated group (group5) showed a significant increase in SIRT-1level, significant decrease in DRP-1 level as compared to the valproate treated group (group3), indicating amelioration of mitochondrial dysfunction, significant increase in SIRT-1 level, significant decrease in DRP-1 level as compared to risperidone treated group (group 4) in both cerebellum, and hippocampus. Combination group (group6) showed a significant increase in SIRT-1level, significant decrease in DRP-1 as compared to the valproate treated group(group3),significant increase in SIRT-1, significant decrease in DRP-1 level as compared to risperidone treated group (group 4), and non-significant difference in SIRT-1 and DRP-1 level as compared to celastrol treated group (group 5) in both cerebellum, and hippocampus.

Celastrol ameliorated apoptosis in VPA-induced ASD:

VPA-treated group (group3) revealed a significant increase in caspase level as compared to the normal control group (group1) in both cerebellum, and hippocampus. Risperidone treated group (group4) revealed non-significant difference in caspase level as compared to the valproate treated group (group3) in both cerebellum, and hippocampus. Celastrol treated group (group5) showed a significant decrease in caspase level as compared to the valproate treated group (group3),indicating improved apoptosis, significant decrease in caspase level as compared to risperidone treated group (group 4) in both cerebellum, and hippocampus. Combination group (group6) showed a significant decrease in caspase level as compared to the valproate treated group (group3), significant decrease in caspase level as compared to risperidone treated group (group 4), and non-significant difference in caspase level as compared to celastrol treated group (group 5) in both cerebellum, and hippocampus.

Celastrol improved inflammation in VPA-induced ASD:

VPA-treated group (group3) revealed a significant increase in IL-6 level as compared to the normal control group (group1) in both cerebellum, and hippocampus. Risperidone treated group (group4) revealed a significant decrease in IL-6 level as compared to the valproate treated group (group3) in both cerebellum, and hippocampus. Celastrol treated group (group5) showed a significant decrease in IL-6 level as compared to the valproate treated group (group3) in both cerebellum, and hippocampus, denoting improved inflammation. Combination group (group6) showed a significant decrease in IL-6 level as compared to the valproate treated group(group3), significant decrease in IL-6 level as compared to risperidone treated group (group 4), and significant decrease in IL-6 level as compared to celastrol treated group (group 5) in both cerebellum, and hippocampus.

Celastrol ameliorated oxidative stress status:

VPA-treated group (group3) revealed a significant decrease in GSH level, a significant increase in MDA level as compared to the normal control group (group1) in both cerebellum, and hippocampus. Risperidone treated group (group4) revealed non-significant difference in GSH and MDA level as compared to the valproate group (group3) in both cerebellum, and in hippocampus. Celastrol treated group (group5) showed a significant increase in GSH level, a significant decrease in MDA level as compared to the valproate group (group3), a significant increase in GSH level, significant decrease in MDA level as

compared to risperidone treated group (group 4) in both cerebellum, and hippocampus. Combination group (group6) showed a significant increase in GSH level, a significant decrease in MDA level as compared to the valproate group (group3), a significant increase in GSH level, a significant decrease in MDA level as compared to risperidone treated group (group 4) and non-significant difference in GSH and MDA level as compared to celastrol treated group (group 5) in both cerebellum, and hippocampus.

Table-I:Effect of risperidone, celastrol and the combination of both on different measured parametersin VPA-rat animal model of autism. Results expressed as mean± SEM of 6 groups (8 rats each).

Groups / Parameter		Group1 Control	Group 2 Vehicle	Group 3 VPA	Group 4 Risperidone	Group 5 Celastrol	Group 6 Combination
Three chamber test (session-I)	Length of time in empty chamber	2.3 ± 0.4	3.0 ±0.7	8.0 ± 0.5	3.9 ± 0.5	4.2 ± 0.5	2.2 ± 0.2
	Length of time spent with stranger-1	7.7 ±0.4 +	7.0 ±0.7 +	2.0 ± 0.5 #+	6.1 ± 0.5 * +	5.8 ± 0.5 *+	7.8 ± 0.2 *+
Three chamber test (session-II)	Length of time spent with stranger-1	2.4 ± 0.3	2.9 ± 0.2	7.5 ± 0.4	3.9 ±0.2	3.9 ± 0.4	3.5 ± 0.4
	Length of time spent with stranger-2	7.6 ±0.3 +	7.1 ±0.2 +	2.5 ± 0.4 #+	6.1 ± 0.2 *+	6.1±0.4 *+	6.5±0.4 *+
T maze test (percentage of alternation %)		95.0 ±3.3	90.0 ±3.8	27.5 ± 3.7 #	57.5 ± 4.5 *&	65.0±5.0 *&	87.5±5.3*
Tissue SIRT-1	Cerebellum	10.5± 0.6	9.9± 0.8	1.1± 0.1#	3.5± 0.8 &	8.1± 0.7 *	10.1 ± 0.4 *
	Hippocampus	9.9± 0.3	9.3± 0.4	1.4±0.2 #	3.1±0.5 &	7.5±0.5 *	8.8±0.4 *
Tissue DRP-1	Cerebellum	176.0 ± 10.8	183.7 ± 10.2	410.1 ± 8.8 #	357.0± 20.4 &	235.1±12.7 *	181.9 ± 10.1 *
	Hippocampus	164.9 ± 8.4	168.3± 10.1	388.8 ± 16.4 #	342.5±21.7 &	230.0 ±18.5 *	184.5 ± 2.7 *
Tissue caspase-3	Cerebellum	0.18 ± 0.08	0.36 ± 0.09	1.7 ± 0.09 #	1.4± 0.2 &	0.75 ± 0.08 *	0.32± 0.13 *
	Hippocampus	0.11± 0.04	0.32±0.09	1.5± 0.05 #	1.1± 0.14 &	0.44±0.13 *	0.17±0.05 *
Tissue Interleukin-6	Cerebellum	92.6 ± 4.4	117.0 ± 7.9	257.9± 6.1 #	145.1± 6.5 *&	128.0 ± 7.6 *&	95.3± 6.6 *
	Hippocampus	82.0±3.4	105.5±7.9	246.5± 5.8 #	138.6± 6.1 *&	119.3±7.7 *&	92.8±3.1 *
Reduced glutathione (GSH)	Cerebellum	5.4 ± 0.3	4.7 ± 0.2	2.7 ± 0.2 #	3.0±0.2 &	4.8 ± 0.2 *	5.4±0.1 *
	Hippocampus	4.6 ± 0.2	3.7±0.2	2.1± 0.2 #	2.6±0.2 &	3.9 ± 0.3 *	4.3 ± 0.2 *
Malondialdehyde (MDA)	Cerebellum	15.5±0.4	24.4 ± 1.1	61.3 ±5.2 #	48.1±8.3 &	28.3 ± 2.7 *	16.6± 0.9 *
	Hippocampus	13.9±0.8	21.2±0.7	53.5± 5.4 #	43.1± 7.4 &	25.9±1.5 *	15.6±1.1 *

Celastrol improved histopathological findings in VPA-induced ASD in both cerebellum and hippocampus (Fig.2):

Histopathology in the VPA-treated group (group 3) Showed diminished number of purkinje cells with altered cerebellar structure (Fig.2C), numerous neuronal degeneration and chromatolysis in hippocampus indicating VPA-induced apoptosis in neurons (Fig.2D). Risperidone treated group (group 4)Showed diminished number of purkinje cellswith altered cerebellar structure (Fig.2E), neuronal degeneration and chromatolysis (Fig.2F). celastrol treated group (group 5)Showed apparently normal cerebellum with intact Purkinje cell layer (Fig.2G), normal hippocampal architecturewith minimal degeneration (Fig.2H). Combination group (group 6)Showed apparently normal cerebellum with intact Purkinje cell layer (Fig.2I), normal hippocampal architecturewith minimal degeneration (Fig.2J).

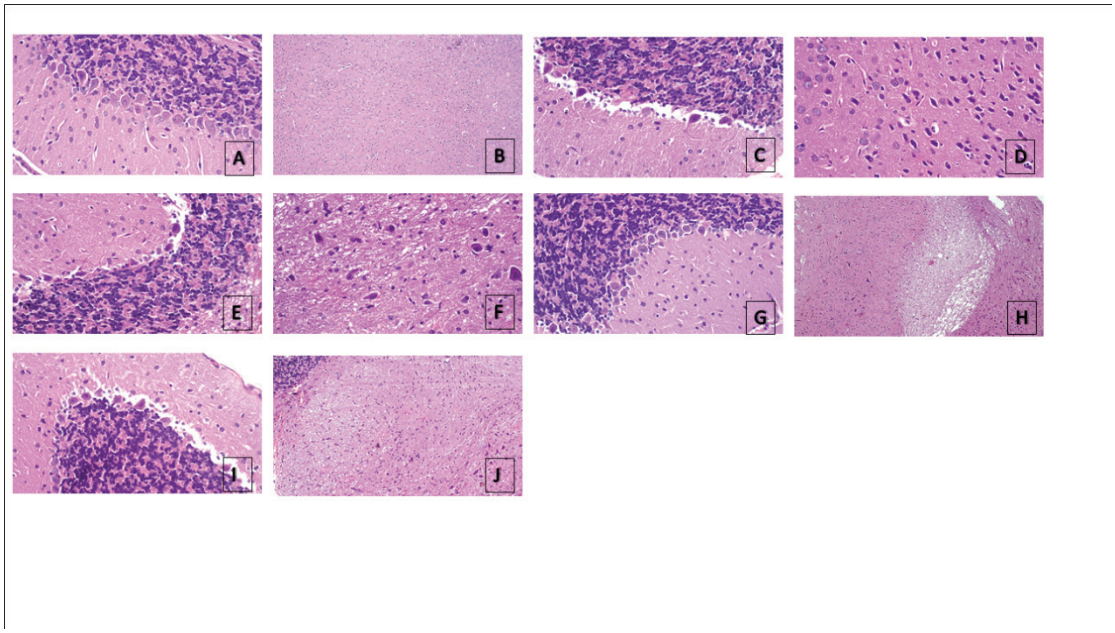

Fig. 2; Histopathology in the control group (group 1) (A) Showing normal cerebellum with intact Purkinje cell layer (H&E X400), (B) Showing normal hippocampal architecture (H&E X100). Histopathology in the VPA-treated group (group 3) (C) Showing diminished number of purkinje cells (arrows) with altered cerebellar structure (H&E X400), (D) Showingnumerous neuronal degeneration and chromatolysis in hippocampus indicating VPA-induced apoptosis in neurons (H&E X400). Histopathology in Risperidone treated group (group 4) (E) Showing diminished number of purkinje cellswith altered cerebellar structure (H&E X400), (F) Showingneuronal degeneration and chromatolysis (H&E X400). Histopathology in celastrol treated group (group 5) (G) Showing apparently normal cerebellum with intact Purkinje cell layer (H&E X400), (H) Showing normal hippocampal architecturewith minimal degeneration (H&E X100).Histopathology in combination Risperidone and Celastrol group (group 6) (I) Showing apparently normal cerebellum with intact Purkinje cell layer (H&E X400), (J) Showing normal hippocampal architecturewith minimal degeneration (H&E X100).

Discussion

Recently, many studies confirm that a significant proportion of individuals with autism have mitochondrial disease. The prominent epigenetic regulatory role of SIRT-1 (silent information regulator-1) in controlling mitochondrial function may underlie its recently reported neuroprotective effect in numerous neurological diseases[24]. SIRT-1 is a histone deacetylase that control PGC1α (peroxisome-proliferator-activated-receptor c coactivator 1α) which is the key regulator of mitochondrial biogenesis[25], PGC1α is found to be highly expressed in cells rich in mitochondria as neurons especially newly generated neurons in embryonic as well as early postnatal life. Regarding mitochondrial dynamics, mitochondria undergo continuous remodeling by growth and fission of each mitochondria, two key proteins are responsible for mitochondrial fission dynamin-related protein 1 (DRP1) and fission 1 protein (Fis 1).

The results of the present study substantiate the idea that mitochondrial dysfunction plays an important role in ASD, animals in the valproate treated group presented a state of mitochondrial dysfunction in CNS as represented by significant decrease in SIRT-1 and significant increase in DRP-1 level. Mitochondrial dysfunction results in oxidative stress that further aggravates mitochondrial impairments, as ROS produced inside mitochondria induce DRP-1 causing mitochondrial fission culminating in vicious circle that eventually results in initiating apoptotic cascade leading to neuronal cell death[26]. VPA induce an imbalance between oxidative stress and antioxidant system, reduced glutathione (GSH) is the main cellular free radical scavenger in the brain and this oxidative stress state leads to neuronal damage, In the present study, valproate treated group presented a state of oxidative stress in both cerebellum and hippocampus presented bysignificant decrease in reduced glutathione content and marked increase in lipid peroxidation represented by significant increase in MDA and this result agree withRossignol et al[27].

Regarding caspase-3 level, valproate treated group exhibited a significant increase in caspase-3 in both cerebellum and hippocampus. This result agree with apoptotic results in ASD obtained by El-Ansary et al[28].

In the present study, animals in valproate treated group showed a significant increase in IL-6 level, VPA induce microglial activation, active microglia increased production of the pro-inflammatory mediator IL-6 causing neuronal damage and loss, this result is supported byMasi et al[29].

In absence of a specific treatment for core symptoms of ASD and the many side effects of risperidone especially with long term use, there is an urgent need to search for and find other safe drugs for long-term periods.

To the best of our knowledge, there is no previous researches investigated the effect of celastrol in VPA-rat animal model of autism. In the current study, regarding behavioral tests celastrol ameliorated social deficits induced by VPA as manifested in three chamber test by significant increase in length of time with stranger-1 compared to time spent at empty chamber at session I, indicating improved sociability.as well as it showed preference for the chamber containing a newly introduced animal (Stranger 2) over a chamber containing an already familiar animal (Stranger 1) evidenced by significant increase in length of time in minutes spent with stranger-2 compared to time spent with stranger-1 at session II , indicating increased social motivation and novelty in celastrol treated group.as well as there was a significant increase in percentage of alterations(%) in T-maze test indicating decrease in repetitive/ restricted behaviors. Regarding neurochemical results, celastrol treated group showed significant increase in SIRT-1, GSH while it showed

significant decrease in DRP-1, caspase-3, IL-6 and MDA level as compared to valproate treated group. In our study, when celastrol treated group compared to that treated by risperidone, celastrol treated group showed an ameliorating effect on mitochondrial dysfunction as it cause significant increase in SIRT-1 and significant reduction in DRP-1.

These findings suggest that combination of risperidone and celastrol provide an additional amelioration on the disease activity with exhibited additional effects as regard to improvement of behavioral impairment, mitochondrial dysfunction and inflammation. In addition to the additional anti-apoptotic and antioxidant effects of celastrol which provide more neuroprotective influence.

Conclusion

These encouraging results pave the way for using celastrol as an adjuvant therapy during long-term clinical use of risperidone which provide better results and to avoid its neurotoxic impact. This should be verified in further human clinical studies.

Conflict of Interest: None.

Funding: None.

Ethical Clearance: The handling of animals and all experimental procedures were adopted by the institutional "Research Ethics Committee, REC", Faculty of Medicine, Tanta University, Egypt (Approval no. 32548/09/18).

Abbreviations:ASD: Autism spectrum disorder;**DRP-1**: Dynamin-related protein 1; **Fis-1**: Fission 1 protein; **GSH**: Reduced glutathione; **IL-6:** Interleukin-6; **IP**: intraperitoneal; **MDA**: Malondialdehyde; **PGC-1α**: Peroxisome-proliferator-activated-receptor c coactivator 1α; **PND**: Post-natal day; **ROS:** Reactive oxygen species; **SIRT-1:** Sirtuin-1; **TW:**Tripterygiumwilfordii; **VPA:**Valproic acid.

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