NEUROPROTECTIVE EFFECT OF ADIOL AGAINST LITHIUM PILOCARPINE INDUCED-SEIZURES, BEHAVIRAL CHANGES AND COGNITIVE DEFICITESIN MALE MICE

Heba Ahmed Hassan, Dalia Mohamed Abd El motteleb, Nevertyty Mohamed Mahmoud and Mohamed Mohamed Shehata

Faculty of Medicine, Zagazig University, Department of Clinical Pharmacology, Sharkia, Egypt

Corresponding author:

Heba Ahmed Hassan

Email: drhebahassanpharma58@gmail.com

ABSTRACT

Background: ADIOL (5-androsten-3β, 17β-diol) is a metabolite of dehydroepinadro-sterone (DHEA) with antioxidant, anti-inflammatory and antiapoptotic effects. **Objectives**: examine the possible neuroprotective effect of ADIOL against lithium pilocarpine (li-pilo) induced-seizures, behavioral changes and cognitive deficits Methods: 120male mice were divided into six groups, each contain 20 mice which subdivided equally to A for behavioral tests and B for biochemical tests. "Vehicle Group I", "Control Group II "li-pilo", Valproate (VPA) group III (17 mg/kg,i.p). "ADIOL group IV" ADIOL 0.5 mg/kg, "ADIOL group V" ADIOL 1.5 mg/kg "ADIOL group VI" ADIOL 3.5 mg/kg. The animals were observed for the occurrence of spontaneous recurrent seizures (SRS). Open field test, dark light box, acceleration rotarod test and T maze spontaneous alternation were performed. Six mice from each group were euthanized 30 days after pilo injection. Their brains were removed for assessment of oxidant/antioxidant status, anti-inflammatory and antiapoptotic parameters **Results**: li-pilo produced seizures associated with behavioral changes and cognitive deficits, significant decrease in brain superoxide dismutase (SOD) activity and significant elevation in the brain malondialdehyde (MDA), interleukin1β (IL-1β), nuclear factor-kappa B (NF-KB) and caspase-3 (cap-3) in relation to vehicle group. VPA suppressed seizures concomitant with improvement of behavioral changes but worsen short term memory, significant increase in brain SOD activity and significant decrease in MDA, IL-1β, NF-KB and cap-3 levels in relation to li-pilo model. ADIOL (0.5 to 1.5 and 3.5 mg/kg) produced dose dependent decrease in seizures associated with significant improvement of behavioral changes and cognitive deficits, significant increase in brain SOD activity and significant decrease in MDA, IL-1β, NF-KB and cap-3 levels in relation to li-pilo model. **Conclusion:** ADIOL protects against li-pilo induced seizures, behavioral changes and cognitive deficits due to antioxidant, anti-inflammatory, antiapoptotic effects.

Key words: ADIOL, lithium pilocarpine, Seizures, valproate.

INTRODUCTION

Epilepsy is a chronic neurological disorder characterized by repeated spontaneous seizures it is often accompanied by behavioral changes and cognitive deficient [1]. The currently used antiepileptic drugs provide asymptomatic anti-seizure effect without effective prophylaxis or cure. Furthermore, a wide range of adverse effects are associated with their long term use and limit their compliance [2].

Therefore, a need for new antiepileptic drug with better compliance is a promising target.

ADIOL is a major metabolite of dehydroepiandrosterone (DHEA) [3] with previously reported antioxidant, anti-inflammatory [4,5] and anti-apoptotic activities [6]. Several studies have also demonstrated the neuroprotective effects for ADIOL in many neuro-degenerative conditions such as Parkinson's disease and 3-NP-induced neurotoxicity in rat [6,7]. ADIOL can mediate

immunosuppressive effect through ERβ, in which ADIOL bind to ERβ-C-terminal binding protein (CtBP) pathway leading to modulate the extent and duration of inflammatory responses [8].

Behavioral changes and cognitive deficient associated with seizures may be caused by underlying structural lesions and effect of seizures and epileptic foci which discharge impulses affect brain function [9].

Increased inflammatory mediators (IL-1β, NF-κB) and cap-3 associated with elevation in oxidative stress are observed following epileptogenic insult and are thought to be implicated in seizure development[10], possibly by modulating GABA and glutamate homeostasis[11] and blood brain barrier (BBB) breakdown that leads to leakage of albumin and IgG into the brain[12]. Albumin via activation of the transforming growth factor β (TGF)-β pathway, leads to increased neural excitability through down-regulation of inward rectifier potassium channel (Kir4.1) and glutamate transporter [1]. The current work aimed to study the possible protective effect of ADIOL in versus VPA against li-pilo induced-seizures, behavioral changes and cognitive deficites and to investigate the possible mechanism(s) of action.

MATERIALS AND METHODS

Animals

120 adult male Swiss albino mice 8 weeks old weighing 15–35 g were used in the current study. Animals were purchased from the Faculty of Veterinary Medicine, Zagazig University, Egypt. Mice were allowed standard pellet diet and tap water before, during one week of acclimatization period, and through the whole experimental period. They were kept at a constant temperature (23 ±2°C), humidity (60 ±10%) and a light/ dark (12 h: 12 h) cycle. The animals were randomly assigned to experimental groups. Each mouse was used only once and all tests were performed between 8.00 and 15.00 h. The experiment was performed in the pharmacology department laboratory, faculty of medicine, Zagazig University. Experimental design and animal handling were performed in accordance with protocols approved by the local experimental ethics committee guidelines of the Egyptian Society of Neuroscience, the Ethical Committee of the Faculty of Medicine, Zagazig University, for Animal Use and the guidelines of the US National Institutes of Health on animal care.

Experimental protocol

Induction of spontaneous recurrent seizures (SRS) by repeated low dose pilocarpine protocol (RLDP) [13], Li.(127 mg/kg,i.p.) injected one day before pilo, Followed by injection of Methylscopolamine bromide (1 mg/kg, i.p.) (30 min) directly before administration of pilo, then the mice received repeated injections of pilo. (10 mg/kg, i.p) every 30 min until they developed convulsive seizures, at stage 4 or 5 according to [14]. Stage 4 of seizures was characterized by turn over into side position, and clonic–tonic seizures. In stage 5, rats turned into back position, and showed generalized clonic–tonic seizures. Thus, in both stages, there appeared clonic–tonic convulsions.The animals received up to four injections of pilo.(maximum), or until they developed SE. SE was blocked with administered of xylazine (0.1mg/kg, i.m.) [15]. All experimental animals received injections of 5 ml 0.9%NaCl (i.p), directly after SE and twice on the following day after SE to prevent dehydration. The percentage of animals at each seizure stage was calculated in each group [16]. Subsequently, the behavior of animals was videotaped and analyzed 24 h a day (7days a week, for 2weeks, beginning 2weeks after pilocarpine injection), for the occurrence of spontaneous recurrent seizures (SRS). After this time, li-pilo model animals were considered epileptic if it presented at least one episode of SRS during the whole observation period [17].

A-Behavioral tests

4 Weeks after pilo injection we did behavior tests with 1week separate from each test. Open field test for assessment of exploration, dark light box for assessment anxiety, acceleration rotarod test for assessment motor coordination function and T maze spontaneous alternation for assessment working memory.

1-Open field test

A rectangular box (30x50 x50 cm), whose floor was divided into 9 small rectangles. Each animal was released in the center of the arena and allowed to explore it for 5 minutes. The number of line crosses and the frequency of rearing are usually used as measures of

locomotor activity (number of squares crossed), exploration and anxiety. Grooming behavior (licking of body or paw) [18]. Defecation and urination are often used as measures of anxiety [19].The open field was uncontaminated with a 5% water-alcohol solution before behavioral testing to eradicate possible bias due to smells left by previous mice.

2-Dark light box test

The test apparatus consists of a box divided into a small (one third) dark chamber and a large (two thirds) brightly illuminated chamber. The box is measured (42 x 21 x 25 cm) [20]. A restricted opening 3 cm high by 4 cm wide connects the two chambers. A mouse is allowed to move freely between the two chambers for 5 min [21]. After each trial, both chambers are cleaned with70% ethanol from defecation and urination of mice. The first latency to enter the dark compartment and the total time spent in lit compartment are indices for anxiety in mice. Transitions are index of exploration.

3-Accelerating rotarod test:

The mice were placed on the already revolving beam facing the opposite orientation so that forward locomotion was necessary in order to avoid a fall. The latencies before falling for four trials per day for 3 days, each trial lasting 1 min, with a 10-min intertrial interval [22]. The latency to fall was measured by the Rotarod timer.

4-T-maze spontaneous alternation

Each animal was placed in the starting arm with a sliding door blocking the access to the choice arms. The trials started when the experimenter lifted the door and ended when the animal made a choice and was blocked in one of the arms; entrance was recorded when the animal entered, all four paws, in the selected arm. The first trial was a forced-choice trial where either one of the arms was blocked. The subsequent 14 trials were free-choice trials with both arms open and the mouse was allowed to choose. After a choice, the animal was confined in the selected arm until it made contact with the closed door,

which marked the end of the trial. Animals had a maximum time of 2 minutes to perform each trial; after that the animal was gently pushed to the start arm. The T-maze was cleaned with alcohol at 25% between trials to avoid the presence of odour cues. The latency of first entry on the arm and the percentage of alternation were manually recorded [23].

B-Biochemical assays

Six mice from each group were euthanized by decapitation 30 days after pilo injection [16]. Their brains were quickly removed in liquid nitrogen. The whole brain was homogenized in ice-cold saline for estimation of SOD, MDA, IL-1β, NF-κB, cap-3 were estimated utilizing enzyme linked immunosorbant assay (ELISA) kits purchased from (mybiosource, USA), (life spane bioscience, USA),(CUSABIO Bender Med Systems, USA), (EIAab, USA) and (CUSABIO, USA), respectively and results were expressed as Microgram/Gram(u/g) for SOD, picograms per gram tissue (pg/g tissue) for IL-1β, NF-KB and cap-3 and as nanograms per gram tissue (ng/g tissue), for MDA level.

Statistical Analysis

The obtained Continuous variables were tabulated as means± SEM. Comparison between different groups were made using one way analysis of variances (one-way ANOVA) followed by Post-Hoc (least significant difference "LSD") tests as described by **Armitage and Berry** [24]. The categorical variables were expressed as a number percentage. Percent of categorical (ordinal variables were compared using Chi-square test for trend).The differences were considered to be significant when $p < 0.05$. All other results are carried by Graph Pad prism software version 5.

RESULTS

Pharmacological results

In li-pilo model, administration of VPA and ADIOL (0.5, 1.5 and 3.5 mg/kg) protected 95%, 80%, 95% and 100% of mice respectively from reaching stage 4 seizures ($p <0.05$) (Table1).

Tabel (1): Percentage of reduction of stage 4 seizures induced by valproate (17mg/kg, i.p.), ADIOL (0.5, 1.5 and 3.5g/kg, s.c.) in li-pilo model in mice

	Control	Valproate	ADIOL 0.5	ADIOL 1.5	ADIOL 3.5
Percentage	**15%**	**95%[a]**	**80%[a]**	**95%[a]**	**100%[ab]**

Values are means of 6 mice ±SEM. [a] P <0.05 compared to control group, [b] P <0.05 compared to VPA group

A-Behavioral tests results

1- Open field(table 2)

Regarding ambulation frequency, Control group produced significant increase (p<0.05) in ambulation frequency in comparison to vehicle. While VPA (17mg/kg) and ADIOL (1.5 and 3.5mg/kg) produced significant increase (p<0.05) in ambulation frequency in comparison to control group. While, ADIOL 0.5 mg/kg was produced insignificant change in ambulation frequency in comparison with control group.

Regarding no of rearing, Control group produced significant decrease (p<0.05) in mean number of rearing when compared to vehicle group. Even so with valproate (17mg/kg) was produced significant increase (p<0.05) in mean number of rearing when compared to control group. While ADIOL (0.5, 1.5 and 3.5mg/kg) were produced insignificantly change (p>0.05) mean number of rearing in the open field test when compared to control group.

Regarding number of grooming, control group produced insignificant change (p>0.05) in mean number of when compared to vehicle, But valproate (17mg/kg) and ADIOL (0.5 and 1.5 mg/kg) produced insignificant change (p>0.05) in mean number of grooming when compared to control group and vehicle group. While ADIOL 3.5 mg/kg produced significantly increase (p<0.05) in mean no of grooming as compared to control group but, insignificant change (p>0.05) in mean number of grooming when compared to vehicle group.

Regarding number of fecal boli, control group produced significant decrease (p<0.05) in mean number of fecal boli when compared to vehicle. While, valproate (17mg/kg) and ADIOL (0.5, 1.5 and 3.5 mg/kg) produced significant decrease (p>0.05) in mean number of fecal boli when compared to control with no significant change in compared with vehicle group.

Table (2): Effects of VPA (17mg/kg), ADIOL (0.5, 1.5 and 3.5mg/kg) on the mean values ± SEM on exploratory behaviors in open field test (ambulation frequency, no of rearing, no of grooming and no of fecal boli) in the mice li-pilo model.

Group N=6	Vehicle	control	Valproate	ADIOL 0.5	ADIOL 1.5	ADIOL 3.5
Ambulation frequency	2.8 ±77.3	25.8[a]±2.13	76.5± 3	66.6[a]±7.87	98.6[a]±17.8	107.7±14.2
No Rearing	38.6±5.8	7 [a] ±2.3	34.6 [b] ±7.8	17±7.35	17.6±4.85	26.3±7
No Grooming	12.6±2.04	7±1.9	8.6±0.98	4.33±0.95	5.6±1.08	26.3 [b] ±7.08
No Fecal boli	2.5±0.34	7 [a] ±1.4 B	2.33 [b] ±1.3	1.5±0.34	0.83 [b] ±0.4	0.83 [b] ±0.4

Values are means of 6 mice ±SEM. [a] P <0.05 compared to vehicle group, [b] P <0.05 compared to li-pilo

control group
2-Dark light box test (table 3)
Regarding number of transition, control group produced significant decrease (p<0.05) in mean number of transition when compared to vehicle group. Also valproate (17mg/kg) and ADIOL (0.5, 1.5 and 3.5mg/kg) could not produce any significant change (p>0.05) in mean number of transition in relation to control group. Also valproate (17mg/kg) and ADIOL (3.5mg/kg) insignificantly (p > 0.05) decrease mean no of transition as compared to vehicle group. While, ADIOL 0.5 and 1.5 mg/kg significantly (p<0.05), decreased mean no of transition in comparison with vehicle group.

Regarding number of time that mice spent in dark compartment in sec, control group produced significant change (p<0.05) in mean dark time when compared to vehicle group. But valproate (17mg/kg) and ADIOL (0.5, 1.5 and 3.5mg/kg) could not produce any significant change (p>0.05) in mean dark time when compared to control group or vehicle.

Regarding number of time that mice spent in light compartment in sec, control group produced significant decrease (p<0.05) in mean light time when compared to vehicle group. But valproate (17mg/kg) and ADIOL (0.5 and 1.5mg/kg) could not produce any significant change (p>0.05) in mean light time in when compared to control group or vehicle group. Furthermore, ADIOL (3.5mg/kg) had produced significant increase (p<0.05) in mean light time when compared to control group by with no significant change (p>0.05) with vehicle group.

Table (3): Effects of VPA (17mg/kg), ADIOL (0.5, 1.5 and 3.5mg/kg) on the mean values ± SE on exploratory behaviors in dark light box test (no of transition, mean dark time and mean light time) in mice lithium pilocarpine model.

Group	vehicle	control	Valproate	ADIOL 0.5	ADIOL 1.5	ADIOL 3.5
Mean No of Transition	8.6±1.2	2 [a] ± 0.5	4.3± 0.5	2.3 [a] ±0.4	3.6 [a] ±0.8	6.3±2
Mean Dark Time (sec)	171.7±12.7	272.7 [a] ±5.6	233.3±14.4	201.7±21.9	218.7±29.3	200.5±21.7
Mean Lit Time (sec)	30.5±5	4.5±1.8	43.6±9.3	38.3±14.8	65.8±27	61.8[b]±21.7

Values are means of 6 mice ±SEM. [a] P <0.05 compared to vehicle group, [b] P <0.05 compared to li-pilo control group

3-Accelerating rotarod test (table 4)
Regarding the latency to fall from the rotarod bar, control group produce no significant change (p>0.05) in mean mean latency period when compared to vehicle group. While valproate (17mg/kg) significantly (p<0.05) increased mean latency period as compared to control group, with no significant difference (p>0.05) from vehicle group. Furthermore, ADIOL (0.5 and 1.5 mg/kg) was insignificantly (p>0.05) decreased mean latency to fall from as compared to control group, with no significant difference (p>0.05) from vehicle group. Also ADIOL (3.5 mg/kg) significantly (p<0.05) increased mean latency period in comparison to control group.

Table (4): Effects of VAP (17mg/kg) and ADIOL (0.5, 1.5 and 3.5mg/kg) on the mean values ± SE on mean latency to fall in sec in rotarod test (3 days) in mice lithium pilocarpine model.

Group	vehicle	control	valproate	ADIOL 0.5	ADIOL 1.5	ADIOL 3.5
Mean latency To fall in sec	39.6±3.6	25.2± 3.5	55.3 [b] ± 4.1	33.5±2.54	38.9±2.7	52.9 [b] ±5.9

Values are means of 6 mice ±SEM. [a] P <0.05 compared to vehicle group, [b] P <0.05 compared to li-pilo control group

4-T-maze spontaneous alternation (table 5)

Regarding spontaneous alternation, control group produced significant decrease (p<0.05) in % of spontaneous alternation when compared to vehicle group. While, valproate (17mg/kg) were insignificantly (p>0.05) increased the mean % of spontaneous alternation as compared to control group, it is still insignificantly (p>0.05) lower than vehicle group. Also ADIOL (0.5and 1.5 mg/kg) were insignificantly (p>0.05) increased mean % of spontaneous in relation to control group. These were insignificantly (p>0.05) lower than vehicle group. While, ADIOL (3.5 mg/kg) was significantly (p<0.05) was mean % of spontaneous with control group and It was insignificantly (p>0.05) lower than vehicle group.

Regarding the Latency in the starting arm, control group produced significant decrease (p<0.05) in mean Latency in the starting arm when compared to vehicle. Valproate (17mg/kg) produced significant (p<0.05 decrease in mean Latency in the starting as compared control group, it is still insignificantly (p>0.05) lower than vehicle group. Also ADIOL (0.5, 1.5 and 3.5 mg/kg) were significantly (p<0.05) decreased mean Latency in the starting arm in comparison with control group. These were significantly (p<0.05) higher than vehicle group.

Table (5): Effects of VAP (17mg/kg) and ADIOL (0.5, 1.5 and 3.5mg/kg) on the mean values ± SE on % of spontaneous alternation and latency to first entry in start arm in spontaneous alternation T-MAZE test in mice lithium pilocarpine model.

Group	vehicle	control	valproate	ADIOL 0.5	ADIOL 1.5	ADIOL 3.5
% Spontaneous alternation	90.9±2.3	61.8 [a] ± 13.1	77± 5.4	88.9±5	86.4±1.9	90.2 [b] ±3.8
Latency to first Entry in start Arm	10.8±1.2	23.83 [a] ±4.9	12.5 [b] ±0.7	12.1 [b] ±0.8	17.6 [b] ±4.85	10.8 [b] ±0.6

Values are means of 6 mice ±SEM. [a] P <0.05 compared to vehicle group, [b] P <0.05 compared to li-pilo control group

Biochemical results

Compared to vehicle animals, li-pilo mice produced significant reduction (p<0.05) in brain SOD activity by 84.2%. Moreover, epileptic mice showed significant (p<0.05) increase in brain level of MDA, IL-1β, NF-κB and cap-3 by 547.3%, 459.2%, 860% and 522.2%, respectively (figure1: A,B,C,D,E). Mice which pretreatment with VPA for 2 days before pilo produced significant (p <0.05) increase in brain SOD activity by 373.3%,Furthermore, VPA significantly (p<0.05) decreased brain level of MDA, IL-1β, NF-KB and cap-3 by 78%, 76.1%, 79.3% and 72.9%, respectively as compared to li-pilo control group(figure1A,B,C,D,E). ADIOL(0.5mg/kg) for 2 days before pilo produced significant (p <0.05) increase in brain SOD activity by 66.6%,also produce significant (p<0.05) decrease in brain level of MDA, IL-1β, NF-κB and cap-3 by 36.5%, 41.7%, 37.5% and 38.7%, respectively as compared to li-pilo control group (figure1A,B,C,D,E). While with pretreatment of epileptic mice with ADIOL(1.5mg/kg) for 2 days before pilo produced significant (p <0.05) increase in brain SOD activity by 166.6% associated with significant (p<0.05) decrease in brain MDA, IL1β, NF-κB and cap-3 by 51.4%, 55.6%, 56.3% and 54.2%, respectively as compared to li-pilo control group (figure1A,B,C,D,E). with increasing dose of ADIOL to (3.5 mg/kg),animals produced significant (p <0.05) increase in brain SOD activity by 300% concomitant with significant (p<0.05) decrease in brain MDA, IL-1β, NF-KB and cap-3 by 67.6%, 65.5%, 73.3% and 69.8%, respectively as compared to li-pilo control group(figure1A,B,C,D,E)
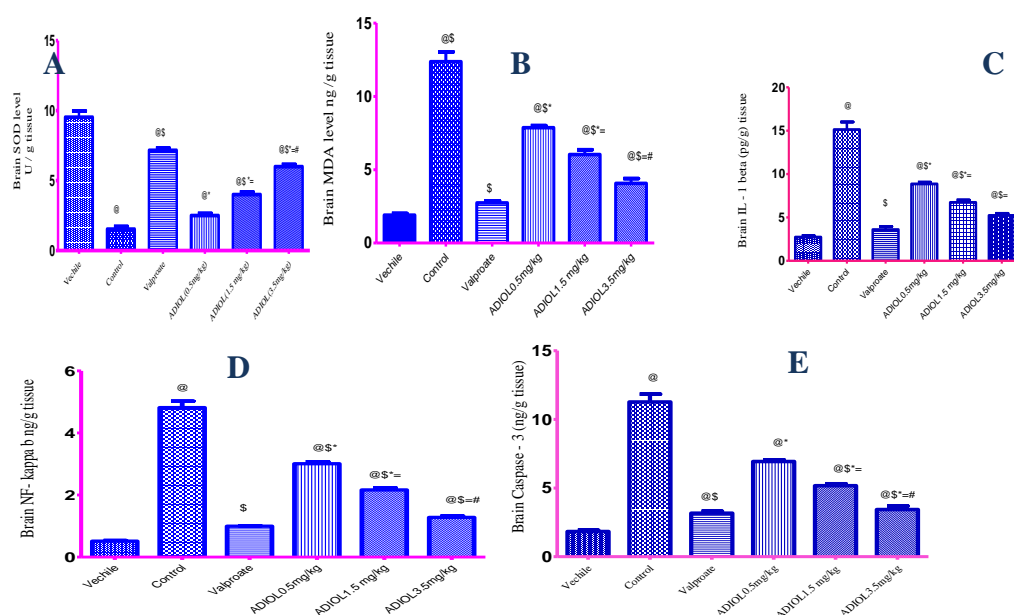
Fig. 1: showing comparison between different groups regarding brain SOD, MDA, IL-1β, NF-KB and cap-3.Values are means of 6 mice ±SEM. @ P <0.05 compared to vehicle group, $ P <0.05 compared to li-pilo control group, *P <0.05 compared to valproate group, =P <0.05 compared to ADIOL 0.5 group, #P <0.05 compared to ADIOL 1.5 group.

DISCUSSION

The results of the current study revealed that 85% of mice subjected to RLDP reached stage 4/5 clonic convulsions and were associated with significant tissue reduction in SOD activity with significant increase in MDA level in brain mice. These results are in agreement with those of Ahmad et al[25]; Armenta et al. [26], who reported high levels of MDA and low SOD activity in brain of epileptic rate which induced by li-pilo.

It is postulated that oxidative stress resulting from excessive free-radical release is likely implicated in the initiation and progression of epilepsy [27,28] Oxidative stress occurs during epileptogenesis due to seizures-associated glutamate excitotoxicity and NMDA receptor overactivation [29] leading to increase in oxidation of macromolecules of the neurons just before the neuronal loss [30]. In its turn, free radicals inhibit glutamine synthase (which transforms glutamate into glutamine inside astrocytes), inhibit glutamate decarboxylase (which converts glutamate into GABA) and inactivate glutamate transporter causing increase glutamate concentration and diminish GABA level [31]. Also, ROS may activate NFκB, leading to the production of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α, which in turn enhance inflammation and, therefore, the generation of more reactive species [32,33].

In the current study, i.p injection of li-pilo significantly elevated brain level of pro-inflammatory cytokines; IL1β and NF-κB levels. These findings are in agreement with Yang et al. [34] who found that serum levels of IL-1β, NF-κβ and TNF-α in children with MTLE were significantly higher than the vechile as well as in animal models of epilepsy induced by chemoconvulsants or by electrical stimulation [35,36,37].

The possible explanation could be seizure activation caused neuronal cell loss and the destruction of the BBB [38], associated with increasing inflammatory responses and neural excitability in the brain [39]. IL-1β and NF-KB produced by astrocytes leads to the activation of the PI3K/Akt/mTOR signaling pathway after combining with the interleukin-1 receptor (IL-1RI) on the neurons, and that the activation of this signaling pathway plays an important part in the pathogenesis of MTLE[40]. cytokines contribute to epileptognesis in multiple ways, by increasing neuronal hyperexcitability via blocking astrocyte-mediated reuptake of glutamate from the synaptic space [35], also enhance NMDA receptor function[41] with alternation of GABAergic neurotransmission [42]. Also modulates voltage-gated ion channels and

contributes to channelopathies [43]. NF-κB activation leads to activation of non-transcriptional pathway [44] which cause phosphorylation of voltage-dependent and receptor-coupled ion channels, thereby directly affecting neuronal excitability and seizure threshold [36].

The present study showed significant elevation of caspase 3 level in brain mice which injected with li-pilo. This work coincided with **Khadrawy et al.** [45] who reported elevation of cap3 level in rat brain after induction of SE by li-pilo. **Fujikawa** [46] suggested that SE leads to excessive activation of NMDA receptors by glutamate, allowing calcium influx through their receptor-operated cation channels causing excitotoxicity [47] resulting in downstream swelling and rupture of intracellular organelles and activated proteolytic enzymes leading to cell death [48]. NF-κB activation with epilepsy can also induce the generation of neurotoxic free radicals; resulting in neuronal apoptosis [49]. The active caspase 3 is responsible for the cleavage of key intracellular structural and survival proteins and activates the enzyme responsible for the DNA fragmentation [50].

The Present study shows, VPA administration protects against li-pilo induced SE with reducing brain oxidative stress, hampered brain level of inflammatory mediators and protect brain cell from apoptosis. According to **Rajeshwari et al**. [51], the antioxidant effect of VPA is attributed to its free radical scavenging ability, enhancement of enzymatic antioxidant network (SOD, catalase and glutathione peroxidase). In addition, VPA was reported to elevate the level of non-enzymatic antioxidants namely, vitamin C, vitamin E, and reduced glutathione, which scavenge the residual free radicals escaping from decomposition by the antioxidant enzymes [52]. VPA block NMDA receptor and Na^+ channel-mediated glutamate release and excitotoxicity in cerebral nerve endings [53] which may be an additional contributing factor in ROS production inhibition. As a HDACs inhibitor, VPA has the ability to activate the expression of genes targeting antioxidant enzymes (e.g. SOD), and induce several neuroprotective proteins such as angiogenin, brain-derived neurotrophic factor (BDNF), endothelial cell growth factor (ECGF1), and ganglia cell-derived neurotrophic

factor (GDNF) [54,55] The anti-inflammatory effect of VPA is due to modulation of ion channels (Na^+, K^+, Ca^{2+} and Cl^-) in immune cells leading to suppression of cytokines production [56,33]. **Himmerich et al.** [33], reported that the cytokine production is due to elevation in ROS generation. Thus, the anti-inflammatory effect of valproate may be due to antioxidant effect. VPA exhibits dose-dependent biphasic effect on apoptosis of activated lymphocytes probably through differential modulation of several apoptosis-related signaling pathways as VPA in low dose had anti-apoptotic effect through activation of extracellular signal-regulated kinases (ERK) pathway and phosphorylation of anti-apoptotic molecules such as Bcl-2 [57] associated with significant elevation of phosphorylated STAT3 lead to the attenuation of activation-induced apoptosis , but at high doses it promoted apoptotic cell death by induction of p38 mitogen-activated protein kinase (MAPK) and mitochondrial apoptosis pathway with increase dephosphorization of STAT3 resulting in enhanced activation-induced apoptotic cell death [58,59].

This work demonstrated that, pretreatment of epileptic mice with ADIOL produced significant dose dependent antiepileptic effect associated also with dose dependent antioxidant, anti-inflammatory and antiapoptotic effects.

These results are matched with **Reddy and Jian** [60]; **Carver and Reddy** [61] they stated that ADIOL caused dose-dependent suppression of seizures in mouse hippocampus kindling epilepsy. This could be explained by **Clossen and Reddy** [62], who stated that, ADIOL (a neurosteroid) induced changes in neuronal excitability, through modulation of GABAARs ([63] which permit influx of chloride ions into the cell causing hyperpolarizing of the membrane and reduced excitability at cellular and systems level [64]. As ADIOL at low concentrations, act as positive allosteric modulator, potentiating Cl^- influx by increasing the duration and frequency of channel opening [60,61] but at higher concentrations, can directly activate the GABAARs [65,61].

In agreement with our study, **Hanna et al**. [6] reported that ADIOL (25 mg/kg, s.c.) for two days before 3- 3-nitropropionic acid displayed antioxidant, anti-inflammatory and antiapoptotic

activities as evidenced by the elevation of cortical and striatal reduced glutathione levels, reductions of cortical and striatal MDA, striatal TNF-α and IL-6 levels. Only a small number of iNOS and caspase-3 positive cells were detected in sections from rats pretreated with ADIOL. Also **Salama et al**. [7] stated that ADIOL in doses 0.35, 3.5 and 35 mg/kg decreased striatal and nigral NF-κB level in rotenone induced-parkinson's disease rat model with reduced cap3, Bax, Bax/Bcl level. Also ADIOL inhibited the expression of IL-1β upon LPS stimulation of human microglia and the expression of iNOS mRNA in mouse astrocytes when cells were stimulated by IL-1β [67].

Saijio et al. [67] explained that ADIOL functions as a selective modulator of (ERβ) to suppress inflammatory responses of microglia and astrocyte by mediate recruitment of ERβ-C-terminal binding protein (CtBP) corepressor complexes to Activator protein-1which is transcriptional factor responsible for gene expression for cytokines and growth factors [68]., leading to suppressing genes that amplify inflammatory responses and activate Th17 T cells. Reduction of endogenous ADIOL or ERβ expression results in exaggerated inflammatory responses to TLR4 agonists. ADIOL promote CtBP recruitment prevents experimental autoimmune encephalomyelitis in an ERβ-dependent manner. These findings provide evidence for an ADIOL/ERβ/ CtBP-transrepression pathway that regulates inflammatory responses in microglia and can be targeted by selective ERβ modulators [67].

In both O.F and LDT, control group exhibited decrease in exploratory behavior with increase anxiety behavior in relation to vechile group.These results are consistent with [69,70] who reported that, reduced locomotor and exploratory behavior associated with increased in anxiety behavioral in li-pilo model, was observed in the Irwin screen, open field, hole-board, light-dark box. As SE induced by li-pilo causes extensive neuronal loss in the hippocampus, cortex, amygdala and thalamus [71]. These structures are known to be functionally important in anxiety and exploration [72,73], also damage to them produce behavioral impairment.

But pretreatment of mice with VPA, animals increase in exploratory behavior and decrease in anxiety behavior but in LDT no difference was detect in the behavior of mice in relation to control group. In agreement with current study **Zádori et al.** [74] stated that VPA significantly increased the ambulation distance (no of crossed square) and increase in spontaneous locomotor activity in open field test in in a transgenic mouse model of Huntington's disease. Due to VPA increases GABA levels in the substantia nigra pars reticulate which is an important part of both direct and indirect pathways of the basal ganglia circuitry leading to decrease inhibition of the thalamus, resulting in increased locomotion

With pretreatment of mice with ADIOL 0.5 mg/kg exhibited no effect on exploratory behavior but significant decrease in anxiety behavior while with increase dose to 1.5 and 3.5mg/kg animal exhibit significant increase in exploratory behavior and significant decrease in anxiety behavior. But in LDT no difference was detect in the behavior of mice in relation to control group except for ADIOL 3.5 mg/kg decrease anxiety behavior. In line with our results, **Salama et al.** [7] showed that pretreatment of rotenone-induced Parkinson disease rat model rate by ADIOL 3.5mg/kg increase in no of crossed squares also ADIOL 3.5 and 35 mg/kg showed significant improvement in the exploratory and increase in rearing frequency in rat due to increased DA level with significant decline in DA turnover. Also, **Tsutsui and Haraguchi** [75] reported the modulatory effects of neurosteroids on DA in pineal body which stimulates locomotor behavior. Also ADIOL is a selective modulator of ERβ [67]. **Borrow and Handa** [76]reported that ERβ had anxiolytic effect which observed in pharmacological studies utilizing selective ERβ agonists as diarylpropionitrile caused decreased anxiety behavior in rats and mice in a number of tests, including the open field test, elevated zero maze test elevated plus maze test, and light–dark box test [77,78]**.** As ER increased the expression of tryptophan hydroxylase (TPH) (the rate-limiting enzyme in serotonin synthesis), this accompanied by increases in the levels of TPH enzymatic activity and total serotonin which act on 5-HT1A receptor leading to anti-anxiety effect [79].

In this study our observations regarding the rotarod test performance show that there is no

significant difference in latency to fall, between li-pilo group and vechile group. This result coincides with **wu et al.** [80] who reported that li-pilo model had no effect on motor coordination (no effect on rotarode test).This work demonstrates that VPA produce significant improvement in motor coordination in comparison with li-pilo group. In agreement with our result **Weihl et al.** [81] who suggested that VPA improve muscle strength and function in patients with type III/IV spinal muscle atrophy by increased transcription of spinal muscular atrophy genes and protein in vitro leading to increase in quantitative muscle strength and subjective function of muscle. Also, **Yu et al.** [82] demonstrated the ability of VPA to improve motor coordination in mice with traumatic brain injury by inhibiting glycogen synthetase kinase 3 (GSK-3) and HDAC effect of VPA leading to reduction of lesion volume and attenuation of blood-brain barrier disruption. Furthermore, **Esteves et al** [83] reported that chronic VPA (200mg/kg) treatment of Mouse Model of Machado-Joseph Disease lead to improvement in the motor performance, given by the beam balance, motor swimming, rotarod due to increase in histone acetylation by its HDACi effect which mediate gene transcription leading to improve ataxic symptoms in a transgenic mouse [84]. ADIOL 0.5 and 1.5 mg/kg produce no significant improvement in motor coordination in comparison with control group but with increasing the dose to 3.5 mg/kg showed significant improvement in motor in comparison to li-pilo group.As ADIOL is a selective modulator of (ERβ) that had significant effect on motor coordination due to its physiological role in cerebellum of adults [85] by preventing neuronal deaths, inducing formation of synapses and increasing information transmission through nerve impulses [86], maintaining the structure [87] and function of cerebellum[86].

With i.p injection of li-pilo to mice, animal showed impairment in memory. These results are agreed with **Hoppe et al.** [88] who stated that patients with MTLE often associated with memory impairment. Also impaired learning and memory is observed in the pilo model of MTLE in rats and mice [89]. Cognitive and behavioral impairment that associated with TLE are resulting from hippocampal sclerosis, Loss of neurons in the hippocampus and associated gliosis [90]

While pretreatment of epileptic mice with VPA, animals show no improvement in memory impairment. Some studies coincide with currant work, as VPA and other anticonvulsant mood stabilizers have generally been found to have some adverse effects on cognition in patients with epilepsy [91]. **Sgobio et al.** [92], reported that VPA induced morphologic alterations and impairment in specific hippocampal-dependent memory task as VPA inhibits acetylcholine esterase (AchE) causing increase acetyl choline in barin leading to cognitive deficits [93]

In present model, pretreatment with ADIOL 0.5 and1.5mg/kg show no improvement in memory impairment but with increasing dose of ADIOL to 3.5 mg/kg produce significant improvement in memory impairment that caused by li-pilo injection.This could be explained by **Bodo and Rissman** [94] who reported the role of ER β in memory ability that vary along the menstrual cycle in women [95,96] and decline in this ability by post-menopausal women which counteracted by estrogen replacement therapy[97].

CONCLUSION

ADIOL protects against li-pilo induced seizures, behavioral changes and cognitive deficits due to its antioxidant, anti-inflammatory, antiapoptotic effect.

ABBREVIATIONS

ADIOL: 5-androsten-3β, 17β-diol; AEDs: antiepileptic drugs; AKT: Protein kinase B (PKB);ANG: Angiogenin; BBB: blood brain barrier; BDNF: Brain-derived neurotrophic factor; cap 3: caspase3; CtBP: ERβ-C-terminal binding protein; DHEA: dehydroepinadrosterone;ECGF1: Endothelial cell growth factor; ELISA: enzyme linked immunosorbant assay; ER stress: endoplasmic reticulim stress ;ERK: Extracellular receptor kinase; ERβ: estrogen receptor beta; GABA: gamma amino butyric acid; GABAAR: gamma amino butyric acid receptor; GDNF: Glial cell-derived neurotrophic factor; GSH: Reduced glutathione; HDAC: histone deacetylase activity; i.m.: intramuscular; i.p: intraperitoneal; I/R: Ischemia reperfusion IgG: immunoglobulin g; IL-1:interleukine 1;IL-1R: IL-1 receptor;IL1β:

inteleukin1β;IL-6: interleukine 6;Kir4.1: inward rectifier potassium channel; li-pilo: lithium pilocarpine LPS:lipopolysaccharides ;MAPK: mitogen-activated protein kinase ;MDA: malondialdehyde; MES: maximal electroshock seizures; MTLE: mesial temporal lobe epilepsy; MTOR; mammalian target of rapamycin; n: number of animals; NF-KB: nuclear factor kappa b; NMDA: N-methyl-D-aspartic acid; ROS: Reactive oxygen species;PI-3-K: Phosphoinositide-3-kinase; PTZ: Pentanyltetrazol ; RLDP: repeated low dose pilocarpine protocol; s.c.: subcutaneous; SOD: superoxide dismutase; SRS: spontaneous recurrent seizures; TGF-β: transforming growth factor β;TLR : Toll like receptor: TNF-α: Tumor Necrosis Factor-α;VPA: valproate.

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