Research Article

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Creatine and α-lipoic acid improved dexamethasone-induced depressive-like behavioral parameters in mice

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ABSTRACT

Corticosterone treatment in mice impairs mitochondrial function, decreases energy production in the brain, and induces depressive-like behaviors. Creatine (Crt) is vital for energy homeostasis in the brain. Alpha-lipoic acid (ALA) improves mitochondrial function and reduces oxidative stress. The aim was investigating the effect of Crt and ALA following dexamethasone (Dexa) induced depression in mice model of despair.

Female mice (22±3 g) were experimented. Dexa (15 mcg/kg, SC) injected for a week, Crt was inserted in animals' diet, and ALA (25, 50 mg/kg) injected IP. After the locomotor test, behavioral parameters of depression, including immobility time during the forced swimming test (FST), and anhedonia during the sucrose preference test were evaluated. No significant changes were recorded during the locomotor test. Dexa increased the immobility time during the FST (154 ± 6.3 s vs control 119±5.5 s, $p<0.05$). Crt 2 %, reduced immobility time (89 ± 7 s vs normal diet 125 ± 4.7 s, $p<0.01$), ALA (50 mg/kg) reduced the immobility time (88 ± 15 vs control 134±8 s, $p<0.05$). While, Crt and ALA co-administration with Dexa reduced the immobility time during the FST. The results of the sucrose preference test were in line with FST, since Crt and ALA increased the sucrose preference when administered together with Dexa.

Improvement of behavioral parameters in mice treated with Crt and ALA clearly indicates their effect on preventing Dexa depressive-like behaviors. Mitochondrial dysfunction must be further evaluated regarding Dexa induced depressive behavior.

Keywords: Depression, Creatine, Alpha-Lipoic Acid, glucocorticoids, dexamethasone

1. Introduction

The mitochondrial respiratory chain enzyme complexes produce an important part of cellular energy through oxidative phosphorylation. Tissues in need with higher energy level, like the brain, have larger number of mitochondria, thus are more susceptible to the reduction of aerobic metabolism(8). Creatine kinase plays a crucial role in the metabolism of brain, it's effective functioning protects cellular adenosine triphosphate (ATP) levels(8). Studies have provided evidences for the mitochondria-related mechanisms of depressive symptoms(9). Evidently, dysfunction of mitochondrial respiratory chain is an aspect related to the physiopathology of mood disorder(10,11).

It has been shown that inducing depression in animal models is accompanied by mitochondrial bioenergetics impairments in brain parts that are critical in the neural interconnection of depression such as the frontal cortex and hippocampus(12). It has been revealed that 21 days restrained stress in rats inhibited the activities of the first complexes of the mitochondrial respiratory chain(13).

Creatine (Crt) is obtained from foods high in protein or biosynthesized endogenously from arginine, methionine, or glycine in the kidney, liver, and the brain(14). Experimental models and clinical trials have shown beneficial effects of Crt supplementation in neurological diseases that are connected to mitochondrial dysfunction, such as Parkinson's disease(14). This could also be relevant for depression disorders according to the evidence that brain mitochondrial aberrations occur in subjects with depression(8). Animal research indicate that Crt exerts neuroprotection against neurotoxins such as 3-nitropropionic acid, and high levels of glutamate by maintaining ATP homeostasis(15). Therefore, Crt may alleviate depressive behavior by buffering the ATP level, thus preventing energy depletion and neuronal death.

Alpha-Lipoic acid (ALA), also known as 1,2-dithiolane-3-pentanoic acid is an endogenous essential cofactor in the mitochondrial complex and catalyzes the decarboxylation of pyruvate and alpha-ketoglutarate. Due to its antioxidant function exogenous ALA is used as a dietary supplement in key regions like Europe, United States and Japan(16). In addition, it has been demonstrated in rats that ALA reverses mitochondrial structural decay associated to age by lowering oxidative stress injuries and improving mitochondrial performance, and ameliorates cognitive dysfunction in memory tasks(17). It has been assumed that ALA may contribute to the treatment of depression by increasing tryptophan availability in the blood(18).

Although psychiatric effects and mood changes following GCs administration appear to be common, these side effects are generally mild and reversible. Prescribing the common antidepressant drugs would expose persons to unnecessary medications, thus introducing an effective complementary medical treatment would be beneficial. Additionally, a major limitation of commonly antidepressant drugs is their long lag period for showing their therapeutic effects, which often takes at least 4 weeks. Based on the assumption that mitochondria might be involved in the pathophysiology of mood disorder, an observation study was conducted in order to evaluate the effect of Crt and ALA following dexamethasone-induced depressive-like behavior in mice.

2. Material and Methods

Animals

Female albino mice weighing 22±3 g (6-8 weeks old) were maintained at room temperature 21± 2 ºC with free access to standard mice chow and tap-water, on a 12-12 h light-dark cycle (lights on at 6 AM). Each study group consisted six animals that were kept together in a cage, and one day before starting the therapy they were placed in the behavior laboratory room for acclimatization, and behavior tests were performed between 8 AM and 1 PM. Animal procedures were completely performed in accordance with guidelines for the Care and Use of Laboratory Animals Issued by The National Ethical Committee (Ethical No: IR.MUI.REC.1396.3.903). Particular attention was paid to minimize animal distress and to reduce the number of animals used in the experiments.

Locomotor test

The locomotor activity of mice was evaluated in advance for each group in an open field (Borj Sanat, Iran) that was divided into 15 zones by red beams. Each mouse was placed in the corner of the arena to freely explore the field for 3 min. Horizontal exploration (number of zone entries) was counted automatically by passing through the beams and vertical

exploration (rears on hind legs) were recorded manually. Finally, total activity that is the sum of horizontal and vertical explorations was calculated.

Forced swimming test (FST)

FST is an animal model that measures despair behavior as an endophenotype of depression in rodents. In a 2-liter beaker glass (diameter 12.5 cm, depth 12 cm) containing 25 ºC water mice were forced to swim for 6 min. After 2 min habituation period, behavior parameters were recorded by a camera at the last 4 min . The immobility time was measured when mouse had no activity except that required to keep its head above the water. Swimming behavior, was measured as the horizontal movement around the beaker; and, climbing behavior, was measured when mouse had upward movements along the side of the beaker(19). At the end mice were carefully dried to avoid hypothermia and returned to their home cage.

Sucrose preference test

This test measured anhedonia as another depression endophenotype in mice. The test was performed in 4 days that started from day 5 of the protocol. on the first day two bottles of sucrose solution (5 %) were placed in each cage, on the next day there was one bottle of sucrose solution and one bottle of water. After the 2 days of habituation period, two bottles containing 100 ml of sucrose solution and 100 ml of tap water were place in each cage after 24 h the amount of sucrose solution and water consumed from each bottle was measured, and the percentage of sucrose solution preference was calculated. Sucrose preference measured less than 65% was considered as a feature for anhedonia(20).

Drugs and diets

Dexa (8 mg/2 ml ampule, Raha Industry, Iran) 15 mcg/kg was injected SC, while control animals received SC normal saline(6). Crt monohydrate (Karen Pharma and Food Supplement, Iran) was added to ground normal mice chow with the percentages of 2 % and 4% and carefully converted to food pallets, control animals had normal chow. Animals were fed with Crt food pallets alone or during treatment with Dexa. ALA (Sigma Aldrich, India), 25 and 50 mg/kg were injected IP, control group received normal saline IP. ALA was administered alone or consecutively with Dexa. In a separate mice group, the concomitant administration of the 2% Crt palate diet followed by ALA 25 mg/kg were evaluated on Dexa induced depression-like behavior. The entire treatment duration was for 7 days and behavioral tests were performed on the following day. Overall, 13 groups of animals were studied each consisted 6 mice (totally 78 mice).

Data processing and statistical analysis

Results are presented as group mean ± SEM and analyzed by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests. P values less than 0.05 were considered significant. The software programs applied for graph making and analyzing data were Excel 2016 and the GraphPad Prizm 6.

3. Results and Discussion

Food consumption and the value of Crt in the diet

Following administrating Crt in mice diet the average food consumption was significantly higher when Crt was inserted in Dexa treated animals ($p<0.001$ compared with the normal group or compared with Dexa alone group) (Table 1). The actual dose of Crt consumed was 3.6 mg/g, and 6.1 mg/g body weight for food diet containing 2%, and 4% Crt respectively, food consumption was higher in animals that were concomitantly injected with Dexa thus Crt dose increased up to 8.6 mg/g.

The effect of Crt alone and together with Dexa and ALA on depressive behavior

By adding Crt to mice diet immobility time during the FST decreased, that was more pronounced with Crt 2% (89±7 s vs control group with a normal diet, 125±4.7 s, $p<0.01$) (Figure 1).

Dexa administration obviously caused depressive-like behavior as immobility time increased up to 154±6 s that was noticeably higher than control animals (119±5.5 s, $p<0.05$). By adding Crt to the animals' diet that were injected with Dexa immobility time significantly declined compared to Dexa fed with normal chow (Crt 2% 107±8 s and Crt 4% 105±6 s, $p<0.001$). ALA, had additive effects to Crt on reducing the immobility time when they were prescribed together (87±8 s vs Dexa alone $p<0.001$). The sucrose preference test results were

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Table 1. Food consumption and the value of Crt in the diet

Groups (n=6)	Daily food intake, mg/g body weight	Crt daily dose, mg/g body weight	Groups (n=6)	Daily food intake, mg/g body weight	Crt daily dose, mg/g body weight
Normal	141±3	0	Dexa	152±1.6	0
Crt 2%	182±19	3.6	Dexa + Crt 2%	242±1.4$^{***, ###}$	4.9
Crt 4%	153±14	6.1	Dexa + Crt 4%	216±8$^{***, ###}$	8.6

Crt was inserted in the diet, normal group ingested plain mice chow. Dexa (15 mcg/kg SC). Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's comparison test. ***p<0.001 compared with the control group. ### p<0.001 compared with Dexa alone group.

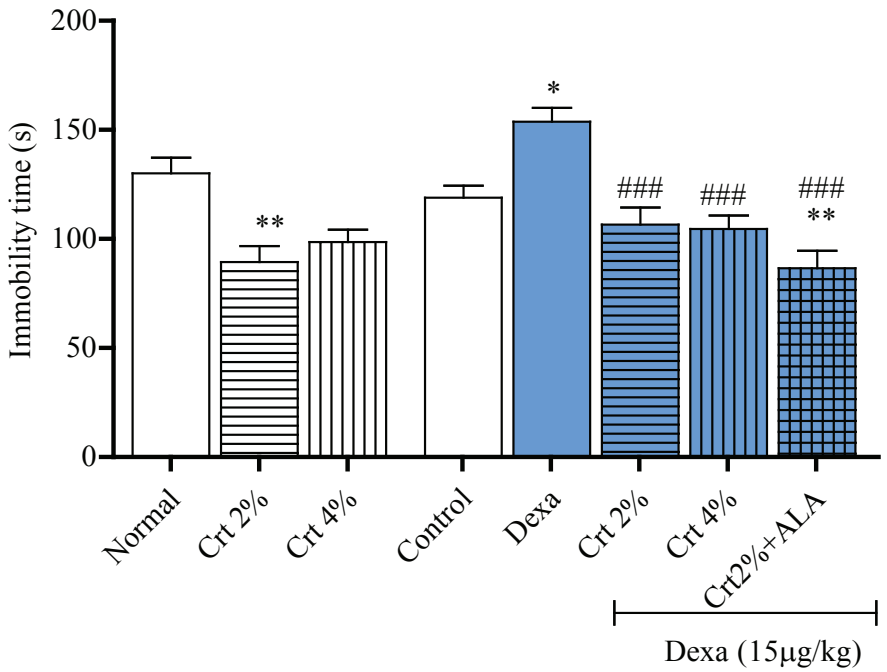

Figure 1. The effect of Crt alone and together with Dexa and ALA on the immobility time during FST. Crt was inserted in the diet, normal group ingested plain mice chow. Dexa (SC) and the control group received normal saline. ALA (25 mg/kg IP). Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's comparison tests (n=6). * p < 0.05, and ** p < 0.01 compared with normal or control groups, ###p <0.001 compared with Dexa alone group.

almost in line with the FST results (Table 2) Dexa has decreased the sucrose preference that is considered as anhedonia, while Crt alone or combined with Dexa has increased the sucrose preference. Table 3 presents animal behavior during the mobile phase of the FST,swimming time is the obvious behavior, Crt ingestion increased it and Dexa injection reduced it although the changes were not significant. Crt 4 % significantly increased climbing time compared to control animals it also increased climbing following Dexa therapy (p<0.05). Combination of the three drugs together significantly increased swimming time. As it is presented in Table 3 the variation of total locomotor activity was not noticeable amongst different groups.

The effect of ALA alone and together with Dexa and Crt on depressive behavior

As attested by Figure 2 AlA injection reduced the immobility time during the FST this change was significant with ALA 50 mg/kg (88±15 s vs control group 134±8 s, p<0.05).

Table 2. Percentage of sucrose preference following different therapies.

Groups (n=6)	Sucrose preference (%)	Groups	Sucrose preference (%)
Normal	69	Control	65
Crt 2%	70	Dexa	47
Crt 4%	79	Crt 2% + Dexa	71
ALA 25mg/kg	59	Crt 4% + Dexa	75
ALA 50 mg/kg	68	ALA25mg/kg + Dexa	74
Dexa + Crt 2% + ALA 25 mg/kg	89	ALA50mg/kg + Dexa	81

Crt was inserted in the diet, normal group ingested plain mice chow, Dexa (15 mcg/kg SC), and the control group received normal saline, ALA injected (IP). Percentage of sucrose preference = (sucrose consumption/ sucrose consumption+ water consumption) ×100

Table 3. The effect of Crt alone and together with Dexa and ALA on the locomotor test and the mobile phase during the FST.

Groups (n=6)	Swimming time (s)	Climbing time (s)	Total locomotor activity
Normal	89±4.6	18±2	137±11
Crt 2%	123±9	26±3	118±12
Crt 4%	110±5	32±3*	164±9.5
Control	93±9.6	16±4	146±13
Dexa	75±5	16±3	131±13
Dexa + Crt 2%	102±9	18±4.3	167±14
Dexa + Crt 4%	102±7	34±3$^{*, \#}$	127±9
Dexa + Crt 2% + ALA 25 mg/kg	127±3$^{*, \#\#\#}$	24±6	170±14

Total activity count during locomotor test = (horizontal +vertical) exploration. Crt was inserted in the diet, normal group ingested plain mice chow. Dexa (15 mcg/kg SC), and the control group received normal saline, ALA injected (IP). Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's comparison test. *P<0.05, compared with the relevant control group, # P<0.05, ###p <0.001 compared with Dexa alone group.

The results were interesting when ALA was injected during Dexa therapy (Figure 2), ALA 25 and 50 mg/kg significantly reduced the immobility time 80±10 s and 78±13 s respectively (p<0.001 vs Dexa alone154±6 s). But the co-administration of ALA with Crt along with Dexa did not further decease immobility time compared with ALA alone. Table 2 shows that ALA 50 mg/kg alone increased sucrose preference percentage and ALA 25 and 50 mg/kg improved the sucrose preference of Dexa when they were co-administrated. Crt and ALA together with Dexa increased sucrose preference to 89%. According to Table 4, swimming time was the important activity during the mobile phase of FST and it was significantly higher in ALA 50 mg/kg plus Dexa group and in the group that Crt was added to the diet (p<0.05 vs control, p<0.001 vs Dexa alone). The climbing time was only higher than control and Dexa alone when ALA 25 mg/kg was administered with Dexa (p<0.01). The table also shows that different therapies did not cause important changes on the locomotor activity.

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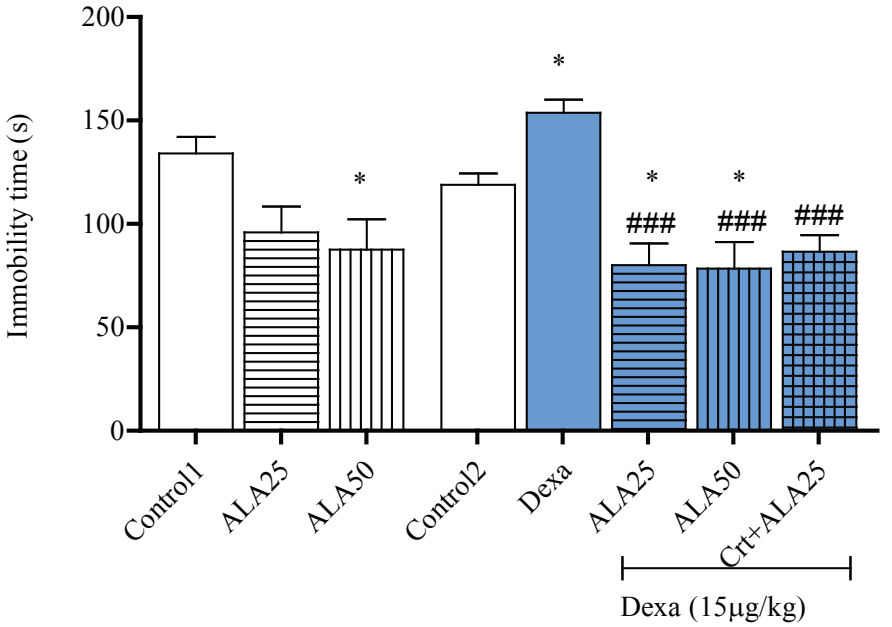

Figure 2. The effect of ALA alone and together with Dexa and Crt on the immobility time during FST. ALA (25, 50 mg/kg, IP), control1 received normal saline. Dexa (SC) control 2 received normal saline. Crt 2% was inserted in the diet. Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's comparison tests (n=6). * $p < 0.05$, compared with control group, ###p <0.001 compared with Dexa alone group.

Table 4. The effect of ALA alone and together with Dexa and Crt on the locomotor test and the mobile phase during the FST.

Groups (n=6)	Swimming (s)	Climbing time (s)	Total locomotor activity
Control 1	88±10	22±7.6	156±14
ALA 25 mg/kg	110±7	35±8.6	124±19
ALA 50 mg/kg	102±11	42±10	142±26
Control 2	93±9.6	16±4	146±13
Dexa	75±5	16±3	131±13
Dexa + ALA 25 mg/kg	108±6	47±6$^{**, \#\#}$	179±18
Dexa + ALA 50 mg/kg	127±11$^{*, \#\#\#}$	35±9	128±12
Dexa + Crt 2% + ALA 25 mg/kg	127±3$^{*, \#\#\#}$	24±6	170±14

Total activity count during locomotor test= (horizontal +vertical) exploration. Dexa (15 mcg/kg SC), ALA (IP), and Crt was inserted in the diet the control groups received normal saline (SC or IP). Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's comparison test. *P<0.05, **P<0.01 compared with the relevant control group, ##P<0.01, ###p <0.001 compared with Dexa alone group.

Discussion

The following research clearly showed that the GC Dexa increased the immobility time in the FST that is an indication for depressive-like behavior in mice. This study for the first time discovered that Crt and/or ALA could improve depressive behavior caused by Dexa, and the results were parallel in both of the behavioral parameters of depression, including immobility time FST, and anhedonia during the sucrose preference test. In the present study female mice were chosen since previously it was shown that Crt effects on depressive behavior in rodents FST are sex-dependent, as female rats displaying more antidepressant-like response(21). FST is an accustomed pre-clinical test for screening the efficacy

of antidepressant substances and to evaluate the effects of neurological modulation on depressive-like behavior(19). The locomotor test is conducted in most psychopharmacological examinations in order to evaluate animal activity in an open field. This test is accompanied prior to the FST test in order to understand the possible sedative or excitatory effects of drugs that could interfere with FST results. The drug therapies in the following research did not affect animal normal locomotor activity.

In agreement with previous studies Dexa increased the immobility time in the FST, which advocates despair and depressive-like behavior, Dexa dose was utilized in accordance with a previous study(6). The sucrose preference test supported depressive-please delete the full stop behavior as the animals did not have any preference to drink sucrose over water which reveals anhedonia. It has been proven previously that corticosterone treatment in mice impairs mitochondrial function, and antioxidant enzymes activity, this could decrease the energy production and increase oxidative stress in the brain and induce depression-like behaviors(22). In addition, Dexa treated animals clearly ingested more Crt diet, that could be a direct interaction of Dexa with the edible Crt diet. Previously, it was shown that by replacing high fat diet with standard chow the mice display behavioral accompanied by increased motivation for palatable foods(23).

By adding Crt in the diet the immobility time reduced during FST. Crt as a substrate for Crt kinase increases phosphocreatine (PCr) that buffer against ATP depletion and thereby exerts neuroprotective effects(24). ATP is the main energy source in the brain, which is tightly coupled with PCr. Crt kinase catalyzes the reaction of ADP with PCr to produce ATP. Therfore, Crt kinase with the mitochondrion, Crt, and PCr establishes a system that is critical in energy homeostasis in high and fluctuating energy demand organs; such as the brain. PCr leaves the mitochondrion and diffuses to the cytoplasm where it serves as both a temporal and spatial energy buffer(24).In addition to the energy buffering property of Crt that could be important in improving depressive-like behavior Crt effect on the neurotransmitters should also be considered. Measuring swimming and climbing behavior in FST may verify the possible neurotransmitters involved by various therapies. The catecholaminergic agents decrease immobility time while increasing the climbing behavior, and serotonin-related compounds such as fluoxetine decrease immobility time while increasing swimming behavior(19). Crt increased swimming time although the difference did not reach statistical significance compared with the control group, however Crt 4% increased the climbing behavior which may be related to catecholamines modifications. On the downside of the following research, the neurotransmitter levels were not evaluated that is suggested for further evaluation. However previous pharmacological studies support this hypothesis since they have shown that Crt can enhance tyrosine hydroxylase activation, and it can increase brain dopamine level in the substantia nigra by protecting against striatal dopamine depletion in mice model of Parkinson disease(24,25). Also, it was shown that Crt supplementation reduces tryptophan: tyrosine ratio, suggesting that Crt modulates brain serotonin and dopamine level(26). Furthermore, Crt antidepressant effect that was examined by tail suspension test is probably mediated by activation of α1-adrenoceptor since it was ended by prazosin (α1-adrenoceptor antagonist)(27). This was against a previous study which showed females rats fed 2 and 4% Crt performed higher swimming behavior with no differences in climbing behavior(21). Indeed, differences in the study design could be responsible; they used Sprague–Dawley rats and fed their animals for 5 weeks.

Feeding the animals with Crt diet along with Dexa injections prevented the depression-like effects induced by Dexa. The change in climbing behavior was obviously observed with 4% Crt in the diet. The hippocampus is among the most vulnerable of brain regions to neuron loss induced by a seizure, or ischemia hypoxia(28). It is also one of the important GC target sites in the brain, with ample concentrations of GC receptors. GCs cause undesirable effect in the hippocampus, causing massive neuron loss that could be related to depressive behavior(28). It has been advocated previously that the buildup of PCr could help neurons endure ATP levels for a prolonged period of time, especially during energy depletion or stress conditions(24). Therefore Crt has prevented the harmful effects of Dexa on animal depressive-like behavior.

Injecting ALA reduced the immobility time and this change was noticeable with the ALA 50 mg/kg. While the swimming time and climbing time were higher than normal but the differences were not high enough to prove significance. The antidepres-

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sant effect of ALA was assumed more than a decade ago by Salazar(18). Moreover, ALA antidepressant-like effects observed during FST is in agreement with earlier published data(29,30). Pathological effects of oxidative stress should also be considered in psychiatric disorders, such as MDD(31). ALA is a mitochondrial antioxidant and a neuroprotective agent, therefore the effects of ALA in improving depression-like behavior supports the hypothesis that antioxidants could have antidepressant properties(31). Furthermore, ALA had a profound effect on reducing Dexa induced depression. As noted earlier swimming time was increased especially with ALA 50 mg/kg along with Dexa administration although the lower dose of ALA also significantly increased the climbing time. This further support the previous claim that probably ALA increases tryptophan level in the brain(18). Synthesis of serotonin depends on tryptophan concentration in blood (the precursor for serotonin) and tryptophan hydroxylase activity (a rate-limiting enzyme in the synthesis of serotonin). Normally a rise in tryptophan availability results in a rise in serotonin synthesis(32). Therefore, another posibility that must be considered carefully in ALA antidepressant effect would be its effect on serotonin synthesis. Adding ALA to Crt treatment increased Crt antidepressant-like effect. That is ALA has potentiated Crt protecting effect against the harmful effects of Dexa on animal behavior While the combination treatment did not increase the antidepressant effects of ALA alone. further molecular and cellular investigations are warranted regarding Crt and ALA protection against Dexa mitochondrial oxidative damage and their effects on the neurotransmitters.

Conclusion

In general, improvement of behavioral parameters in mice treated with Crt and ALA indicates a clear effect of these two compounds in modulating mood and depressive behaviors. Dexa administration induced depression-like behavior that was reversed by Crt and/or ALA. Therefore mitochondrial dysfunction must be further evaluated as regard to Dexa induced depressive behavior.

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Conflict of Interest

Authors confirm that there is no conflict of interest in relation to this article.

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