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Research Article

Alpha-lipoic acid attenuates depressive symptoms in mice exposed to chronic unpredictable mild stress.

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Alpha-lipoic acid, Depression, Chronic Unpredictable Mild Stress, Immobility time, Behavioural Despair, Oxidative Stress, Serotonin, BDNF

ABSTRACT

Background: Depression is the most common psychiatric illness that involves mood disturbances affecting many brain regions. Despite many approaches available to treat depression, only about 35% of depressed patients achieve remission upon receiving antidepressants. Alpha-lipoic acid (ALA) is an antioxidant that plays an essential role in mitochondrial energy metabolism and neurotransmitter modulation. Hence, this research was aimed at assessing a possible antidepressant effect of ALA in mice exposed to chronic unpredictable mild stress (CUMS). **Methods:** Twenty-five **(**25) Swiss albino mice weighing between 20-26 g were grouped into five groups of five mice each (n=5). Group 1: which served as control received normal saline (NS) and was exposed to CUMS, Groups 2, 3, and 4 received graded doses of ALA (100, 200, and 400 mg/kg respectively), Group 5 (positive control) received fluoxetine (20 mg/kg). Daily administration was done through oral gavage. The animals were subjected to open field (OF) and staircase (SC) tests after induction of depression using CUMS. Thereafter, brain and blood samples of the mice were collected for serotonin, brain-derived neurotrophic factor (BDNF), catalase, superoxide dismutase (SOD), and malondialdehyde (MDA) analysis. **Results**: Treatment with ALA 200 mg/kg significantly decreased immobility time compared to CUMS + NS group ($P \leq 0.05$) in the tail suspension test. Similarly, fluoxetine 20 mg/kg significantly increased brain serotonin level and decreased BDNF level compared to CUMS + NS group ($P \leq 0.05$). However, ALA did not significantly affect brain serotonin and BDNF levels ($P > 0.05$). In the OF test, a significant decrease was observed in the number of line crossings in ALA 100, 200, and 400 mg/kg and fluoxetine 20 mg/kg administered groups when compared with CUMS + NS group ($P \leq 0.05$). However, in SC test and oxidative stress biomarkers, no significant effect was observed ($P > 0.05$). **Conclusion**: ALA showed a promising antidepressant-like effect in mice subjected to CUMS murine model of depression by decreasing immobility time.

INTRODUCTION

Depression is the most common psychiatric illness that involves mood disturbances affecting brain regions such as the hippocampus, temporal lobe, amygdala, caudate, anterior cingulate cortex, and frontal cortex that are involved in a mood-regulating circuit (Jiao-Jie *et al.,* 2016). (WHO, 2020). Depression may result in premature death, major social and economic consequences. Depressed mood, diminished interest/pleasure, alterations in appetite and sleep, and fatigue are among the core symptoms of Major Depressive Disorder (MDD) (Marcia *et al.,* 2016; Muraro *et al*., 2019). In addition to the well-defined depressive symptoms, patients suffering from MDD consistently complain about cognitive disturbances, significantly exacerbating the burden of this illness. Among cognitive symptoms, impairments in attention, working memory, learning, and memory or executive functions are often reported (Flavie *et al.,* 2016).

Several oxidative disturbances in depression have been reported in clinical and preclinical studies, including

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elevated lipid peroxidation levels, decreasing activity of glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), and consequently may contribute to the dysfunction of serotonergic and noradrenergic systems (Marcia *et al.*, 2016). Similarly, MDD is associated with inflammation and mitochondrial dysfunction (Visentin *et al*., 2020). Despite many approaches available to treat depression, only about 35% of depressed patients achieve remission upon receiving treatment with antidepressants, and treatment response rates appear to reduce with each subsequent retry (Hashimoto, 2019; Visentin *et al*., 2020).

Alpha-lipoic acid (ALA) is a naturally occurring compound commonly found in mitochondria, necessary for different enzymatic functions (Bahare *et al.*, 2019). Alpha-lipoic acid was first discovered in 1937 by Snell as an acetate-replacing factor and was characterized by Reed in 1951 (Snell *et al.*, 1937; Reed *et al.*, 1951). Alpha-lipoic acid's first clinical use dates from 1959 in the treatment of acute poisoning by death cap, also known as Amanita *phalloides*, (mushrooms) (Bock *et al.*, 1959; Bahare *et al.*, 2019). Alpha-lipoic acid is synthesized de novo in the body from cysteine and fatty acids in small quantities. Therefore, it is important to consume exogenous sources of ALA to have a therapeutic effect (Victor *et al.*, 2019). Alpha-lipoic acid is widely found in several animal nutritional sources such as bovine kidney, heart, and liver and vegetable nutritional sources such as spinach, broccoli, tomatoes, Brussel sprouts, peas, potatoes, and rice bran (Manuel *et al.*, 2016; Leonardo, 2020).

Physiologically, ALA acts as a cofactor for the α-ketoglutarate dehydrogenase complex to protect mitochondria from oxidative attack (Wei *et al.*, 2019). Both ALA and its reduced form dihydrolipoic acid (DHLA) have a determinant role in oxidative metabolism. For instance, it has been shown that ALA or DHLA has several positive health benefits, including serving as powerful biological antioxidants, metal chelators, and detoxification agents, being also able to reduce the oxidized forms of other antioxidant agents such as glutathione, vitamins C & E, and modulate insulin and nuclear factor kappa B (NF-κB) signaling pathways (Gomes and Negrato, 2014; Moura *et al.*, 2015). Besides, ALA can cross the blood-brain barrier (Choi *et al.*, 2015). Despite the comorbidity of anxiety with depression (Sally *et al.*, 2016), ALA did not significantly increase serum cortisol levels in the rats (Cevik and Aslan, 2015). It has been hypothesized that ALA supplementation in MDD would result in antidepressant effects, possibly via increased insulin sensitization and a consequent increase in tryptophan (precursor of serotonin) synthesis (Salazar, 2000). Following the monoamine hypothesis of depression, Santos *et al.* (2010) found that ALA (20 mg/kg) increased norepinephrine and dopamine levels in the rat hippocampus. However, the same dose of ALA decreased serotonin content and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) levels, and no significant change was observed in the metabolites of monoamines 3,4-hydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) levels in the hippocampus. The possible brain mechanism of action of ALA from these results might be via alterations in hippocampal monoamines providing therapeutic implications in the treatment of neurodegenerative diseases (such as depression). Hence, this study was carried out to assess the possible antidepressant effect of ALA in mice subjected to CUMS.

MATERIALS AND METHODS

Experimental Design and Animal Treatment

Twenty-five (25) healthy 6-8 weeks old Swiss albino mice weighing between 20-28 g were obtained from the Department of Human Physiology, Ahmadu Bello University, Zaria, and allowed access to feed and water *ad libitum* in a normal photoperiod. The animals were singly housed in separate cages (length × width × height: 20×15×10 cm) and assigned into five groups, with five mice each (n=5). Daily administration was carried out based on daily body weight per mouse an hour before the commencement of the experiment which lasted for 2 weeks. Group 1 was exposed to CUMS and received normal saline (10 ml/kg), Group 2 received ALA (100 mg/kg) (Patrocinio *et al*., 2014), Group 3 received ALA (200 mg/kg) (de Sousa *et al.*, 2015; Yusha'u *et al*., 2019), Group 4 received ALA (400 mg/kg) and Group 5 received fluoxetine (20 mg/kg) (Bing *et al.*, 2016). All administration was through oral gavage. Ethical clearance was obtained from Ahmadu Bello University Committee on Animal Use and Care with the approval number: ABUCAUC/2017/003.

Drugs and Reagents

Alpha-lipoic acid was purchased from Puritan's Pride Inc. Ronkonkoma, NY 11779 the United State of America with a Product Code: B68499 02C 14−18988PPS and LOT # T18B045−01 CC1. Fluoxetine was purchased from Bristol Laboratories Ltd., Hertfordshire, the UK with Batch Number: 8775 and product license number: PL11311/0047. Mouse serotonin, BDNF, SOD, CAT, and MDA ELISA Kits were purchased from Shanghai Coon Koon Biotech Co., Ltd. Shanghai, China with LOT # 201907.

Behavioral Assays
All behavioral assays were started at 8:00 hours. The sequence of the assays was as follows: Tail suspension test 1 (TST1) on day 1, chronic unpredictable mild stress on days 2-15, tail suspension test 2 (TST2) on day 16, chronic unpredictable mild stress on days 17-30, tail suspension test 3 (TST3) on day 31, open field test on day 32 and staircase test on day 33.

Chronic Unpredictable Mild Stress
Chronic unpredictable mild stress (CUMS) is a model of depression that exposes mice chronically to constant unpredictable stressors resulting in the development of behavioral changes which cause decreased response to reward and this can only be restored to normal level by chronic treatment with antidepressant drugs (Willner, 2017) and other substances having antidepressant-like activity. These stressors include 45° tilted cage, overnight illumination, white noise, damp sawdust (with 300 ml of water), empty cage, empty cage plus water (300 ml), new clean cage, social stress, and predator (meowing) sound (Zhang *et al.,* 2015). Two different stressors were applied daily (once in the morning and once at night) for one month. In the first two weeks of the experiment, only the stressors were applied, while in the last two weeks the stressors were applied concurrently with the treatments.

Tail Suspension Test
The tail suspension test (TST) was performed as described by Gor *et al.* (2010). Mice were suspended from a metal rod mounted 50 cm above the surface by fastening the tail to the rod with adhesive tape 17 cm on the model of the test which is a bar placed on wood with dimension (55 height x 60 width x 12 cm depth). The duration of the test was 6 minutes and immobility time was recorded. Immobility is defined as the absence of any limb or body movements, except those caused by respiration.

Open Field Test
This is a standard behavioral model that assesses anxiety states in rodents in which the anxious behavior of mice to avoid open, unprotected areas, preference for peripheral areas, along periodic freezing are noted. A reduction in normal behaviors such as rearing and grooming is considered an index of anxiety. The apparatus consists of floor space with the dimension of 40 cm x 40 cm and 30 cm in height. The floor space is divided into 16 squares equally. Each mouse was placed at the center of the apparatus and allowed to explore for 5 minutes. The following parameters were noted: 1) Total number of times the animal crosses the central square 2) Ambulation (total number of squares crossed; 3) Rearing (total number of times the animal stood on its rear paws 4) Frequency of defecation 5) Frequency of urination (Prut and Belzung, 2003; Harish *et al.,* 2015).

Staircase Test
The staircase test consists of placing a mouse in an enclosed staircase with five steps and observing the number of steps climbed and rearing made in a 5-min period (Simiand *et al.,* 1984). A step was climbed only if the criterion was met whereby an animal placed all four paws on the step (Urooj *et al.,* 2016).

Blood Collection
Blood samples were drawn by cardiac puncture and collected in plain tubes. The serum was used for the assessment of catalase, SOD, and MDA activities.

Collection of Brain Tissues
Mice brain tissues were collected at 9:00 hours and prepared according to the method described by Zatta *et al.* (2002) and Habila *et al.* (2012). Treated and control animals were sacrificed by decapitation under anesthesia and brain tissue was immediately removed and placed on an inverted Petri dish on ice. The forebrain was dissected, weighed, and homogenized in 10 ml of a medium containing a solution of 0.1 M sodium phosphate 10 W/V, pH 7.5. The total homogenate was centrifuged at 1000 rev/min in a refrigerated centrifuge for 7 minutes. Aliquots of resulting brain homogenates were stored at -20^0 C until utilization. The supernatants were used for serotonin and BDNF levels determination.

Determination of Brain Serotonin and BDNF Levels
The serotonin and BDNF concentrations were detected by mouse serotonin assay kit CK-bio-16918 and mouse BDNF assay kit CK-bio-15795 (Shanghai, Coon Koon Biotechnology) ELISA, China respectively, according to the manufacturer's protocol. Standards, control, and samples were pipetted into the 48-well plates pre-coated with objective antibody and streptavidin-Horse Radish Peroxide (HRP) wells, and any BDNF and serotonin present was bound by the immobilized antibody respectively. After washing away any unbound substances, enzyme-linked polyclonal antibodies specific for mouse BDNF and serotonin were added to the wells respectively. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells. The enzyme reaction yields a blue product that turns yellow when the stop solution was added. The intensity of the color

measured was in proportion to the amount of BDNF or serotonin bound in the initial step. The sample values were then read off from the standard curve for the corresponding concentrations of BDNF in pg/ml and serotonin in ng/ml respectively.

Determination of Oxidative Stress Biomarkers Levels

Catalase (CAT), SOD, and MDA activities were detected using ELISA kits (mouse CAT CK-bio-15864, SOD CK-bio-16990, and MDA CK-bio-20387 respectively) Shanghai, Coon Koon Biotechnology, China. Serum collected from experimental animals and standards (provided in the kit) was dispensed into the 48-well plate pre-coated with an objective antibody and streptavidin-HRP. Any of the antioxidant enzymes or MDA present was bound by the immobilized antibody respectively. After washing away any unbound substances, enzyme-linked polyclonal antibodies specific for mouse CAT, SOD, and MDA were added to the wells respectively. Following a wash to remove any unbound antibody-enzyme reagents, a substrate solution was added to the wells. The enzyme reaction yields a blue product that turns yellow when the stop solution was added. The intensity of the color measured was in proportion to the amount of CAT, SOD or MDA bound in the initial step. The sample values were then read off from the standard curve for the corresponding levels of CAT and SOD in U/ml and MDA in pg/ml respectively.

Statistical Analysis

Results were expressed as Mean ± SEM. All analysis was done using one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test for multiple comparisons using Statistical Package for the Social Sciences (SPSS) version 23. Values with $p \leq 0.05$ were considered statistically significant. "Graphs were drawn using graph pad prism 8"

RESULTS

Effects of Alpha-Lipoic Acid on Behavioral Despair in Mice Subjected to Chronic Unpredictable Mild Stress

The effect of ALA administration on behavioral despair is shown in Table 1. Administration of ALA (200 mg/kg) significantly (P=0.002) decreased behavioral despair as evidenced by decreased immobility time compared to CUMS + normal saline group (Table 1). However, the higher dose of ALA (400 mg/kg) did not significantly (P=0.897) affect the immobility time compared to the CUMS + normal saline group (Table 1). Similarly, fluoxetine (20 mg/kg) did not significantly (0.105) affect the immobility time compared to the CUMS + normal saline group, F (4, 20) = 5.929.

Table 1: Effects of Alpha-Lipoic Acid Administration on Behavioral Despair in Mice Subjected to Chronic Unpredictable Mild Stress using Tail Suspension Test

Groups	IMMOBILITY TIME (SEC)		
	TST1	TST2	TST3
CUMS +NS	146.2 ± 13	192 ± 8.9	266.7 ± 34.5
(10 ml/kg)	146.8 ± 16.8	202.6 ± 11.6	229.2 ± 8.9
ALA 100 mg/kg	162.6 ± 8	243 ± 15.3	124.4 ± 37.1[a]
ALA 200 mg/kg	113 ± 10.8	189.8 ± 17.3	237.6 ± 22.9
ALA 400 mg/kg	149 ± 5.5	230.2 ± 14.2	181.8 ± 23.4
Fluoxetine 20 mg/kg			

Data presented as mean ± SEM**,** [a] Statistically significant when compared to CUMS + normal saline group at TST3, p<0.05 (n=5), ALA: Alpha-Lipoic Acid, CUMS: Chronic Unpredictable Mild Stress, NS: Normal Saline, TST1: Tail suspension test before exposure to chronic unpredictable mild stressors and treatment. TST2: Tail suspension test after exposure to chronic unpredictable mild stressors for two weeks without any treatment. TST3: Tail suspension test after exposure to chronic unpredictable mild stressors for two weeks and two-week treatment with alpha-lipoic acid.

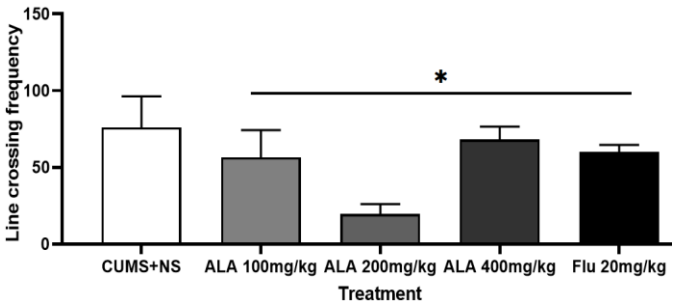

Fig. 1: Line Crossing of CUMS-induced depressed mice. CUMS-induced depressed mice (n=5) per group were treated with graded doses of ALA (100-400 mg/kg), and Flu (20 mg/kg) for 14 days, and thereafter locomotor activity was assessed using OF test. ALA administration decreased locomotor activity in CUMS-induced depressed mice ($p \leq 0.05$). Data were represented as mean ± SEM. *Statistically significant when compared with CUMS + NS group. CUMS: Chronic Unpredictable Mild Stress, ALA: Alpha-Lipoic Acid, Flu: Fluoxetine, NS: Normal Saline.

Effects of Alpha Lipoic Acid on Anxiety-Like Behaviors in Mice Subjected to Chronic Unpredictable Mild Stress using Open Field Test

As shown in Figure 1, ALA (100, 200, 400 mg/kg) and fluoxetine (20 mg/kg) significantly (P = 0.056) decreased locomotor activity as evidenced by decreased line crossing compared to CUMS + normal saline group F (4, 20) = 2.771(Figure 1).

Table 2: Effect of Alpha-Lipoic Acid Administration on Centre Crossing using Open Field Test in Mice Exposed to Chronic Unpredictable Mild Stress

Groups	Frequency of Centre Crossing
CUMS + NS (10 ml/kg)	1.0 ± 1.0
ALA 100 mg/kg	1.0 ± 0.5
ALA 200 mg/kg	0.2 ± 0.2
ALA 400 mg/kg	0.6 ± 0.6
Fluoxetine 20 mg/kg	0.2 ± 0.2

Data expressed as mean ± SEM, p>0.05 (n=5), SPSS version 23, ALA: Alpha-Lipoic Acid, CUMS: Chronic Unpredictable Mild Stress, NS: Normal Saline

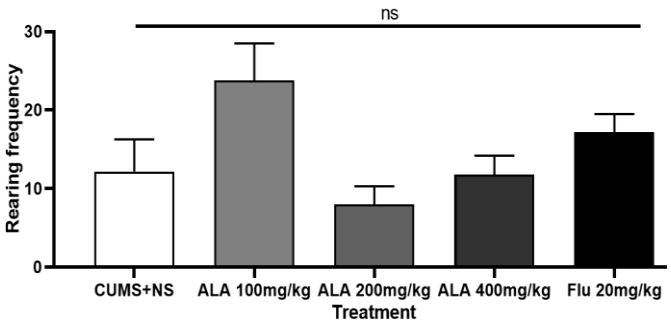

Fig. 2: Frequency of Rearing of CUMS-induced depressed mice. CUMS-induced depressed mice (n=5) per group were treated with graded doses of ALA (100-400 mg/kg), Flu (20 mg/kg), and NS (10 ml/kg) for 14 days, and thereafter, anxiety-like behaviors were assessed using OF test. ALA administration does not improve anxiety-like behaviors in CUMS-induced depressed mice (p>0.05). Data were presented as mean ± SEM. CUMS: Chronic Unpredictable Mild Stress, ALA: Alpha-Lipoic Acid, Flu: Fluoxetine, NS: Normal Saline, OF: Open Field, ns: non-significant.

However, no significant difference (P > 0.05) was observed in center crossing between the treatment groups and CUMS + normal saline group as shown in Table 2. Similarly, administration of ALA and fluoxetine did not significantly (P > 0.05) increase rearing compared to the CUMS + normal saline group as shown in Figure 2. Also, administration of ALA and fluoxetine did not significantly (P > 0.05) change the frequency of defecation and urination when compared to the CUMS + normal saline group as observed in Table 3 and Figure 3 respectively.

Effects of Alpha-Lipoic Acid Administration on Anxiety-Like Behaviors in Mice Subjected to Chronic Unpredictable Mild Stress using Staircase Test.

The effect of ALA administration on anxiety-like behaviors using the staircase test is shown in Figure 4 and Table 4. Groups administered with ALA and Fluoxetine did not significantly (P >0.05) affect the number of stairs climbed (Figure 4) and the frequency of rearing (Table 4) when compared with the CUMS + normal saline group, F (4, 20) = 0.215 and F (4, 20) = 0.619 respectively.

Table 3: Effects of Alpha-Lipoic Acid Administration on Defecation using Open Field Test in Mice Subjected to Chronic Unpredictable Mild Stress

Groups	Frequency of Defecation
CUMS + NS (10 ml/kg)	1.6 ± 0.7
ALA 100 mg/kg	1.8 ± 0.6
ALA 200 mg/kg	1.0 ± 0.0
ALA 400 mg/kg	1.6 ± 0.7
Fluoxetine 20 mg/kg	1.8 ± 0.5

Data expressed as mean ± SEM, p>0.05, (n=5), SPSS version 23, ALA: Alpha-Lipoic Acid, CUMS: Chronic Unpredictable Mild Stress, NS: Normal Saline

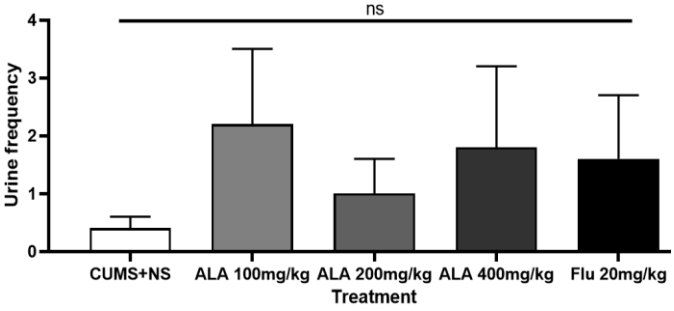

Fig. 3: Frequency of Urination of CUMS-induced depressed mice. CUMS-induced depressed mice (n=5) per group were treated with graded doses of ALA (100-400 mg/kg), Flu (20 mg/kg), and NS (10 ml/kg) for 14 days, and thereafter, anxiety-like behaviors were assessed using OF test. ALA administration does not improve anxiety-like behaviors in CUMS-induced depressed mice (p>0.05). Data were represented as mean ± SEM. CUMS: Chronic Unpredictable Mild Stress, ALA: Alpha-Lipoic Acid, Flu: Fluoxetine, NS: Normal Saline, OF: Open Field, ns: non-significant

Effects of Alpha-Lipoic Acid Administration on Brain Serotonin Level in Mice Subjected to Chronic Unpredictable Mild Stress

As shown in Figure 5, fluoxetine (20 mg/kg) significantly (P = 0.054) increase the brain serotonin level compared to the CUMS + normal saline group. However, the administration of ALA did not show a significant (P >0.05) increase in the brain serotonin level when compared with the CUMS + normal saline group, F (4, 15) = 3.279 (Figure 5).

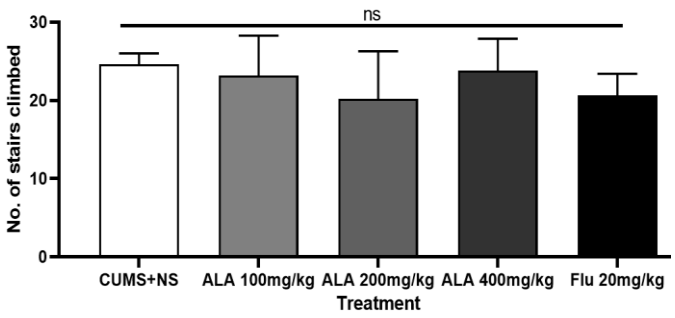

Fig. 4: Number of Stairs Climbed by the CUMS-induced depressed mice. CUMS-induced depressed mice (n=5) per group were treated with graded doses of ALA (100-400 mg/kg), Flu (20 mg/kg), and NS (10 ml/kg) for 14 days, and thereafter, anxiety-like behaviors were assessed using staircase test. ALA administration does not attenuate anxiety-like behaviors in the CUMS-induced depressed mice (p>0.05). Data were presented as mean ± SEM. CUMS: Chronic Unpredictable Mild Stress, ALA: Alpha-Lipoic Acid, Flu: Fluoxetine, NS: Normal Saline, ns: non-significant.

Effects of Alpha-Lipoic Acid Administration on Brain-Derived Neurotrophic Factor Level in Mice Subjected to Chronic Unpredictable Mild Stress

The effect of ALA administration on the BDNF level is shown in Figure 6. Administration of ALA did not significantly (P > 0.05) affect the BDNF level compared to the CUMS + normal saline group (Figure 6). However, a significant (P = 0.056) decrease in brain BDNF level was observed in the group treated with fluoxetine (20 mg/kg) when compared to the CUMS + normal saline group, F (4, 15) = 7.377 (Figure 6).

DISCUSSIONS

The effect of the administration of ALA on CUMS-induced depression in mice was assessed in the present work. Immobility time is an indication of behavioral despair. An increase in immobility time indicates an increase in depressive symptoms, while a decrease in immobility time is an indication of the antidepressant-like effect of a test compound. In this study, ALA (200 mg/kg) reversed the effect of chronic mild stressors by significantly decreasing the immobility time (behavioral despair) at TST3 of the test. This finding depicted that ALA might possess antidepressant-like activity in the mice. This conforms with the hypothesis of Salazar (2000) that ALA might possess antidepressant activity by increasing insulin sensitivity that can lead to an increase in tryptophan absorption with a subsequent increase in serotonin. Similarly, Lin *et al.* (2016) reported that ALA prevented endoplasmic reticulum stress-induced insulin resistance by enhancing mitochondrial functions. Also, Vallianou *et al.* (2009) reported ALA of possessing the ability to increase glutathione (GSH) which helps in decreasing depression via redox-dependent mechanisms of various cellular targets that decrease oxidative stress.

Table 4: Effects of Alpha Lipoic Acid Administration on Rearing in the Staircase Test

Groups	Frequency of Rearing
CUMS + NS (10 ml/kg)	13.0 ± 1.7
ALA 100 mg/kg	16.2 ± 2.9
ALA 200 mg/kg	14.8 ± 4.5
ALA 400 mg/kg	17.6 ± 2.8
Fluoxetine 20 mg/kg	19.4 ± 2.9

Data expressed as mean ± SEM, p>0.05, (n=5), SPSS version 23, ALA: Alpha-Lipoic Acid, CUMS: Chronic Unpredictable Mild Stress, NS: Normal Saline

Table 5: Effects of Alpha-Lipoic Acid Administration on Oxidative Stress Biomarkers in Mice Subjected to Chronic Mild Stress

Groups	SOD (U/ml)	CATALASE (U/ml)	MDA (pg/ml)
CUMS + NS (10 ml/kg)	13.0 ± 8.5	13.0 ± 3.0	1.3 ± 0.6 1.4 ± 0.5 1.9 ± 0.8
ALA 100 mg/kg	9.5 ± 7.1	13.0 ± 1.8	0.8 ± 0.5
ALA 200 mg/kg	20.8 ± 7.7	13.5 ± 0.9	1.5 ± 0.3
ALA 400 mg/kg	13.8 ± 9.2	12.8 ± 1.3	
Fluoxetine 20 mg/kg	16.5 ± 5.9	22.8 ± 8.4	

Data expressed as mean ± SEM, p>0.05, (n=4), ALA: Alpha-Lipoic Acid, SOD: Superoxide Dismutase, MDA: Malondialdehyde, CUMS: Chronic Unpredictable Mild Stress, NS: Normal Saline.

Similar to our findings is the study by de Sousa *et al.* (2015) which showed ALA (200 mg/kg) to have an antidepressant-like effect by decreasing the preference of sucrose and reversing immobility time in the forced swimming test in female Swiss albino mice. Besides, de Sousa *et al.* (2018) found that ALA and desvenlafaxine (DVS), alone or combined, reversed corticosterone (CORT) effects on TST and striatum. This is an indication that ALA can be a promising agent for the treatment of depression and the reversal of cognitive impairment observed in this disorder by reversing CORT-induced memory and social deficits. Furthermore, Santos *et al.* (2010) found that ALA (20 mg/kg) increased norepinephrine and dopamine levels

in the rat hippocampus. However, the same dose of ALA decreased serotonin content and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) levels.

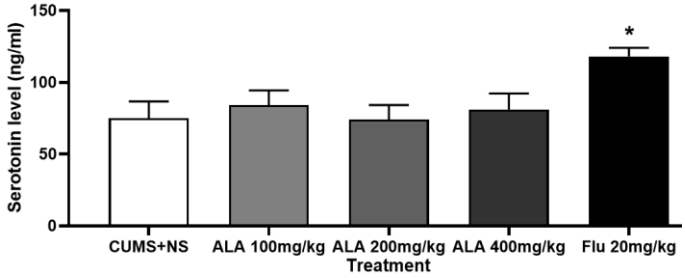

Figure 5: Brain Serotonin Level of the CUMS-Induced Depressed Mice. CUMS-induced depressed mice (n=4) per group were treated with graded doses of ALA (100-400 mg/kg), Flu (20 mg/kg), and NS (10 ml/kg) for 14 days, and thereafter, their brain homogenates were used to assay for serotonin using ELISA kit. ALA administration does not significantly improve the brain serotonin level of CUMS-induced depressed mice (p>0.05). Treatment with Flu significantly improved the brain serotonin level of the mice (p≤0.05). Data were represented as mean ± SEM. *Statistically significant compared with the CUMS + normal saline group at p≤0.05. CUMS: Chronic Unpredictable Mild Stress, ALA: Alpha-Lipoic Acid, Flu: Fluoxetine, NS: Normal Saline.

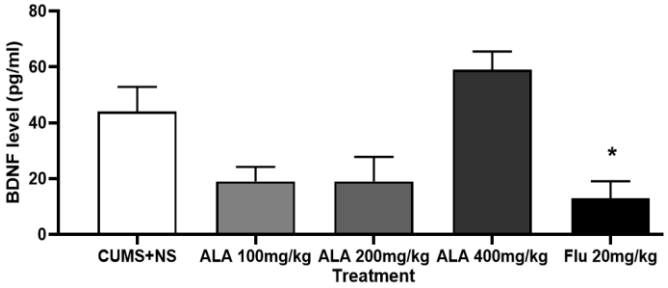

Figure 6: BDNF Level of the CUMS-Induced Depressed Mice. CUMS-induced depressed mice (n=4) per group were treated with graded doses of ALA (100-400 mg/kg), Flu (20 mg/kg), and NS (10 ml/kg) for 14 days, and thereafter, their brain homogenates were used to assay for the BDNF level using ELISA kit. ALA administration did not significantly improve the BDNF level of CUMS-induced depressed mice (p>0.05). Treatment with Flu significantly decreased the BDNF level of the mice (p≤0.05). Data were represented as mean ± SEM. *Statistically significant compared with the CUMS + normal saline group at p≤0.05 CUMS: Chronic Unpredictable Mild Stress, ALA: Alpha-Lipoic Acid, Flu: Fluoxetine, NS: Normal Saline, BDNF: Brain-Derived Neurotrophic Factor.

The possible brain mechanism of action of ALA from these findings might be via alterations in hippocampal monoamines providing therapeutic implications in the treatment of MDD based on the monoamine hypothesis of depression. However, Brennan *et al.* (2013) did not find a significant antidepressant effect of ALA at higher doses of 600-1800 mg/kg in a combination of acetyl-L-carnitine in depressed bipolar human patients. Although, preclinical studies and investigations in individuals affected by Alzheimer's disease provided evidence that ALA may enhance cognition via genetic mechanisms, including mitochondrial activity, antioxidant and anti-inflammatory properties indicating a basis for evaluating ALA's efficacy in MDD as well (Bortalato *et al.,* 2016).

The findings of this study revealed that ALA 100, 200, 400 mg/kg and fluoxetine significantly decrease line crossing (locomotor activity) in OF test. However, Saraswathy *et al.* (2015) reported that ALA did not have a significant effect on locomotor activity in rats using an actophotometer. Our findings depict that ALA is not a psychostimulant and might possess antidepressant-like activity, following the previous report of Cryan *et al.* (2005) who reported that psychotonics are clinically ineffective antidepressants. They show anti-immobility effects in the TST but increase locomotor activity. To detect the possibility of a false-positive result in anti-immobility effects, a locomotor activity test needs to be conducted. Alpha-lipoic acid did not show a significant effect on anxiety-like behaviors in mice exposed to CUMS using OFT and SC in this study. Although anxiety is comorbid with depression (Sally *et al.,* 2016), there is a paucity of literature on the effect of ALA on anxiety in animal models. However, a study on the effect of ALA on serum cortisol level (a biomarker of anxiety) in rats revealed no statistically significant difference between ALA administered group and the control group (Cevik and Aslan, 2015).

Our study showed that ALA (100, 200, and 400 mg/kg) did not significantly increase the brain serotonin level. However, Fluoxetine significantly increased the serotonin level. Santos *et al.* (2010) found that ALA at a low dose of 20 mg/kg decreased rat hippocampal serotonin level 24 hours after administration. Although, we administered ALA for 2 weeks and at higher doses than that in their study. However, ALA in combination with vitamin E reversed the decline in serotonin level of crude homogenate of the whole brain of mice observed in lindane-induced acute neurotoxicity (Renu and Devendra, 2008). Also, Leonardo (2020) reported in a review that treatment with ALA was able to improve the function of the dopamine, serotonin, and norepinephrine neurotransmitters in neurodegenerative disease models. Our findings showing fluoxetine increasing brain serotonin level was widely reported by previous studies. Fluoxetine is a selective serotonin

reuptake inhibitor (SSRI) drug that maintains levels of serotonin in the synaptic cleft by inhibiting the serotonin transporter from attracting serotonin back to the pre-synaptic neuron. This leads to an increase in the level of serotonin in the synaptic cleft available to bind to the postsynaptic receptor (Garnock-Jones and McCormack, 2010; Rachel *et al.,* 2016; Muraro *et al.,* 2019).

In our study, ALA did not significantly affect the brain BDNF level of the mice subjected to CMS. However, De Sousa *et al.* (2015) reported that ALA 200 mg/kg reversed corticosterone (CORT)-induced decrease in BDNF in the hippocampus and striatum of female mice. Besides, administration of ALA 200 mg/kg for 7 days significantly increased BDNF levels in the mice's prefrontal cortex in the CORT-induced depression model (Ramalho-Filho *et al.,* 2014). Fluoxetine significantly decreased the BDNF level of the mice exposed to CUMS in this study. However, Liu *et al.* (2014) found that, in human patients with vascular dementia, Fluoxetine significantly improved serum BDNF level with improved cognitive recovery. Also, the use of antidepressant medication in MDD patients was found associated with increased BDNF mRNA expression in the brain (Bruno *et al.,* 2019). Furthermore, Visentin *et al.* (2020) reported that treatment with antidepressants enhances the synthesis of BDNF and promotes neurogenesis. Therefore, further studies in animal models need to be conducted. Although, it is important to note the limitations of our study such as the small sample size and the BDNF level was assessed in the whole brain, not in some specific areas of the mice brains.

Our study revealed that ALA did not significantly change the serum SOD and catalase levels of the mice. Although, not serum antioxidant levels, a previous study by Veskovic *et al.* (2015) showed that ALA 100 mg/kg IP induced an increase in catalase activity in cortex and striatum and GSH content in the hypothalamus. Moreover, administration of ALA significantly decreased lipid peroxidation and nitrosative stress, caused by methionine and choline deficiency (MCD) diet in all brain regions by restoring antioxidant enzymes activities, predominantly total SOD, manganese superoxide dismutase (MnSOD), and copper and zinc superoxide dismutase (Cu/ZnSOD), and by modulating catalase activity and Glutathione (GSH) content to a lesser extent. However, controversies exist concerning changes in SOD and catalase activities in depressive patients. Interruptions in SOD activity were usually found in depressive patients, but for this disruption, the findings were still inconsistent (Visentin *et al.,* 2020). Reduced SOD activity has been found in MDD patients (Rybka *et al.,* 2013). However, a rise in SOD and catalase activities in depressive patients has been reported in other studies (Kodydkova *et al.,* 2009). Besides, a study showed that serum SOD and catalase activities were significantly higher in the acute phase of MDD patients, showing possibly that increased activities of both antioxidant enzymes might be indicators of acute depressive episodes on MDD (Tsai and Huang, 2016). Alpha-lipoic acid did not significantly affect the serum MDA activity in mice exposed to CUMS in this study. However, Kailash *et al.* (2007) that pretreatment with ALA exerted a very high magnitude of protection against radiation-induced augmentation of lipid peroxidation products (malondialdehyde) and protein carbonyls in mice cerebellum. Similarly, Eman *et al.* (2020) found that the combination of ALA with coenzyme Q10 significantly reduced the MDA level in the cisplatin-induced nephrotoxicity model. Also, Zalejska-Fiolka *et al.* (2015) found that dietary intake of ALA and garlic was significantly associated with reductions of 6-Hydroxydopamine (8OHdG) and MDA levels in rabbits' liver tissue. Besides, Karafakioglu (2019) found that MDA level was observed at the lowest level in ALA 100 mg/kg treated group compared to control and noise exposure groups in a noise-induced oxidative stress rat model.

CONCLUSION

This study demonstrated that ALA exerts antidepressant-like effects on CUMS depressed mice, through a mechanism independent of antioxidant effect, BDNF and serotonin levels. Therefore, ALA is a potential antidepressant drug that can be utilized to attenuate depressive symptoms in depressed individuals. However, in this study, the effect of ALA on the cholinergic system, inflammatory markers, and the norepinephrine system was not assessed. The gene expression of the BDNF and serotonin measured may give a better outcome. Nonetheless, there is a need for further studies on the efficacy of ALA in animal models of depression, cognition, and anxiety.

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