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# Chronic Administration of Alpha Lipoic acid shows Antidepressant-Like Effect in Mice Subjected to Chronic Mild Stress

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## ABSTRACT

Alpha-lipoic acid has been found to increase insulin sensitivity which may lead to increase in tryptophan; a precursor of serotonin. This research evaluated the effect of ALA in mice induced with chronic depression using chronic mild stress (CMS). Fifteen mice were used and grouped into three groups with five mice each. Group I was given normal saline 10 ml/kg, group II and III were given ALA 200 mg/kg and Fluoxetine 20 mg/kg orally respectively. Three tail suspension tests (TST) were conducted (before the chronic mild stress, two weeks of chronic mild stress and after treatment with ALA and Fluoxetine) followed by open field test (OFT) and novel object recognition test (NORT). This study showed that ALA had a significant ($p<0.05$) effect in immobility time (behavioural despair) when compared with Normal Saline group in TST. However, the result of the OFT showed no statistically significant ($p > 0.05$) difference between the control group and the group that received ALA 200 mg/kg in line crossing (locomotor activity). Similarly, ALA did not significantly affect percentage preference in NORT. In conclusion, this study revealed that ALA has an antidepressant-like ability in mice subjected to chronic mild stress.

**Keywords:** *Depression, Alpha-Lipoic Acid, Insulin sensitivity, Locomotor activity, Cognition*

## INTRODUCTION

Depression is a common illness that is a cause of disability worldwide, it affects more than 300 million people of all ages (WHO 2017). Exposure to stressful life events precedes depression and chronic stress results in up-regulation of the production of pro-inflammatory cytokines which has been proposed to be associated with the pathogenesis of depression (Liu et al. 2015). The mechanism associated with depression is not yet fully understood and current treatments remain ineffective in large subset of patients (Menard et al. 2016). The number and severity of episodes determines whether depressive disorder is mild, moderate or severe. Some types of depression show repeated depressive episodes with the patient experiencing loss of interest and enjoyment, diminished activity for at least two weeks, anxiety, sleep and appetite disturbances and feelings of guilt while some type consist of both manic and depressive episodes separated by a period of normal mood. In Africa about 29.19 million people (9% of 322 million) suffer depression with over seven million in Nigeria (3.9 % of 322 million) (WHO 2017).

Alpha-lipoic acid (ALA) also known as 1,2-dithiolane-3-pentanoic acid, is a naturally occurring dithiol

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compound synthesized enzymatically in the mitochondrion from octanoic acid. Alpha lipoic acid is a necessary cofactor for mitochondrial α-ketoacid dehydrogenases, and thus serves a critical role in mitochondrial energy metabolism (Kate et al. 2009). Likewise, ALA is commonly found in dietary components such as vegetables (spinach, broccoli, tomato) and meats, mainly viscera and also in many dietary supplements (Szelag et al. 2012). Due largely to its antioxidant properties, ALA has been reported to afford protection against oxidative injury in various disease processes such as Alzheimer's and obesity (Silva et al. 2013; Gomes and Negrato 2014; Ryota et al. 2015). A number of oxidative disturbances in depression have been reported in clinical and preclinical studies, including 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). Therefore, this study was aimed at assessing possible antidepressant effect of ALA in mice.

## MATERIALS AND METHODS

### Experimental Design and Animal Treatment

Fifteen apparently healthy Swiss albino mice weighing between 20–26 g with ages 6-8 weeks were obtained from Department of Human Physiology, Ahmadu Bello University, Zaria and allowed access to feed and water ad libitum in a normal photoperiod. The mice were grouped into three with five mice per group. Daily administration was carried out based on daily body weight per mouse an hour before the commencement of the experiment for 2 weeks. Group I: Received Normal saline 10 ml/kg. Group II: Received Fluoxetine 20 mg/kg orally. Group III: Received ALA 200 mg/kg orally.Ethical clearance was obtained from Ahmadu Bello University Zaria Committee on Animal Use and Care with approval number: ABUCAUC/2017/003.

### Drugs and Reagents

Alpha lipoic acid was purchased from Novel Ingredient Services, West Caldwell, California, USA with a Product Code: A0030-301 and LOT # 671362552. Flouxetine was purchased from Bristol Laboratries Ltd., Hertfordshire, UK with batch number: 8775 and product license number: PL 11311/0047.

### Chronic Mild Stress

Chronic mild stress is a model of depression that exposes mice chronically to constant unpredictable stressors resulting in the development of behavioural changes which causes 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 bedding, empty cage, empty cage plus water, new clean cage, social stress, and meowing sound (Zhang et al. 2015). Two different stressors were applied daily (one in the morning and one in the night) for a duration of 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 conducted according to the protocol adopted by Adem et al. (2012) and Can et al. (2012). Each mouse was suspended by its tail with an adhesive tape of 17 cm on an aluminum stick placed on top of the apparatus. The dimension of the apparatus is 55 height x 60 width x 12 cm depth. Each mouse was prevented from climbing its tail grasping the side walls by passing the tail through a plastic cylindrical tube. The immobility time (behavioural despair) which is the period where the mice were not moving their body and paws was recorded using a stop watch in a period of six minutes for each animal. The walls of the apparatus were cleaned with 70% ethanol after each trial to prevent any olfactory cue.

The TST was conducted three times; the first one was carried out at the beginning of the experiment, after which the mice were divided into individual cages. The second TST was carried out after two weeks of chronic mild stress without treatments. The third TST was carried out after last two weeks of chronic mild stress with the treatments.

### Open Field Test (OFT)

The apparatus consists of floor space with dimension of 40 cm x 40 cm and 30 cm in height. The floor space was divided into sixteen squares equally. Each mouse was given five minutes to explore the open field arena (test session). Line crossing (an index of locomotor activity) as indicated by the total number of squares crossed was measured during the test session (Harish et al. 2015; Yusha'u et al. 2017).

### Novel Object Recognition Test (NORT)

Novel object recognition apparatus is a rectangular arena that was made of opaque plastic and measured 42 cm × 52 cm. The walls are 40 cm high. Mice were placed in the arena for 5-min where they encountered two identical sample objects (Sample Phase). At the end of the sample phase, mice were placed back in their home cages for a five-minute delay (±15 s). For the testing phase, animals were returned to the arena for 3-min where one of the familiar objects was replaced with a novel object. In addition to the arena, all objects were cleaned with 70% ethanol between each session. Successful

novel object recognition was indexed by greater exploration of the novel compared to the familiar object. The discrimination ratio was calculated as the total time spent exploring the recently seen object (novel object) divided by the time exploring both objects sampled at test (familiar objects) (Thur et al. 2014).

**Statistical Analysis**

The results were expressed as mean ± standard error of mean (SEM). Comparisons between experimental and control groups were performed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc for multiple comparisons with Statistical Package for Social Science (SPSS) version 22. Values with $p < 0.05$ were considered statistically significant.

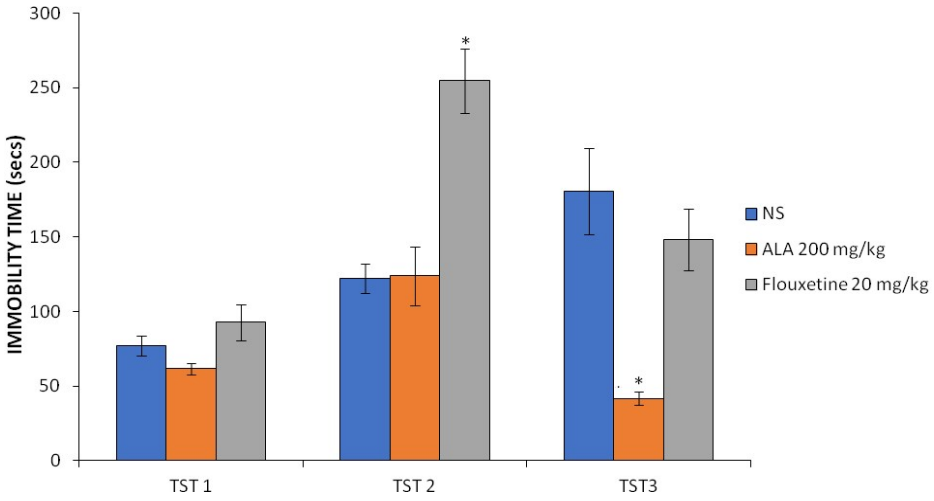


**Fig. 1: Effects of Alpha Lipoic Acid on Depression in Mice Subjected to Chronic Mild Stress.** *The mean difference is statistically significant when compared with Normal Saline $p<0.05$ (n=5), SPSS version 22. TST 1: Tail Suspension Test before exposure of mice to chronic mild stressors. TST 2: Tail Suspension Test two weeks after exposure of mice to chronic mild stressors without any treatment. TST 3: Tail Suspension Test after two weeks treatment with ALA in the last two weeks of chronic mild stress. NS= Normal Saline, ALA= Alpha Lipoic Acid.

**Table 1: Effect of Alpha Lipoic Acid on Locomotor Activity in Mice using Open Field Test**

| Group | Line crossing |
|---|---|
| Normal saline (10 ml/kg) | 114.0 ± 13.59 |
| ALA (200 mg/kg) | 110.4 ± 8.43 |
| Fluoxetine (20 mg/kg) | 89.0 ± 5.12 |

The mean difference was not statistically significant when compared with normal saline group, $p>0.05$ (n=5), SPSS version 22. NS= Normal Saline ALA= Alpha Lipoic acid

## RESULTS

**Effect of Alpha Lipoic Acid on Depression in Mice Subjected to Chronic Mild Stress**

**Assessment of Behavioural Despair in Mice Using Tail Suspension Test before Exposure to Chronic Mild Stressors**

No statistically significant difference was observed in immobility time (behavioural despair) between normal saline group (77.0 ± 6.57) when compared with the other groups; ALA 200 mg/kg and flouxetine 20 mg/kg (61.6 ± 3.89 and 92.8 ± 12.18 respectively), $p = 0.130$, $F (2, 12) = 3.536$. Similarly, no statistically significant difference was observed between fluoxetine 20 mg/kg group and ALA 200 mg/kg group in immobility time, $p = 0.113$ (Figure 1)

**Assessment of Behavioural Despair in Mice Using Tail Suspension Test after Exposure to Chronic Mild Stressors for Two Weeks Without Treatment**

The result of immobility time (behavioural despair) revealed a statistically significant increase between flouxetine 20 mg/kg (254.6 ± 21.59) compared with normal saline group (122.2 ± 10.13), $p = 0.001$. However, no statistically significant difference was observed in immobility time between 200 mg/kg ALA (124.0 ± 19.74) when compared with normal saline group (122.2 ± 10.13), $p = 0.349$, $F (2, 12) = 18.051$ (Figure 1).

**Effect of Two Weeks Treatment with Alpha-Lipoic Acid on Behavioural Despair in Mice Exposed to Chronic Mild Stress using Tail Suspension Test**

The result showed statistically significant decrease in immobility time (behavioural despair) between 200 mg/kg ALA (41.8 ± 4.44) when compared with normal saline group (180.6 ± 28.87), $p = 0.001$. However, no statistically significant difference was observed when flouxetine 20 mg/kg (148.2 ± 20.92) was compared with normal saline group, $p = 0.530$, $F (2, 12) = 12.253$ (Figure 1).

Comparing the TST after treatment with TST before treatment, a decrease in immobility time (behavioural despair) was observed in the ALA 200 mg/kg and fluoxetine 20 mg/kg groups. However, in the normal

saline group an increase in immobility time (behavioural despair) was observed (Figure 1).

**Effects of Alpha Lipoic Acid on Locomotor Activity in Mice Using Open Field Test**

The results of line crossing (locomotor activity) showed no statistically significant difference between the normal saline group (114.0 ± 13.59) and treatment groups ALA 200 mg/kg (110.4 ± 8.43) and Fluoxetine 20 mg/kg (89.0 ± 5.12), p = 0.20. F = (2, 12) = 1.943 (Table 1).

**Effect of Alpha Lipoic Acid on Short Term Memory Using Novel Object Recognition Test**

Result for percentage preference (short term memory) revealed statistically significant difference between Fluoxetine 20 mg/kg (39.8 ± 3.39) when compared with normal saline group (62.5 ± 8.60), p = 0.035. However, no statistically significant difference was observed when ALA 200 mg/kg (58.7 ± 2.82) was compared with normal saline group (62.5 ± 8.60), p = 0.882, F (2, 12) = 4.750 (Table 2).

**Table 2: Effect of Alpha Lipoic Acid on Percentage Preference (Short Term Memory) Using Novel Object Recognition Test**

| Groups | Percentage Preference (%) |
|---|---|
| NS | 62.5 ± 8.60 |
| ALA (200 mg/kg) | 58.7 ± 2.82 |
| Fluoxetine | 39.8 ± 3.39* |

*The mean difference was statistically significant when compared with Normal Saline, p<0.05, (n=5), SPSS version 22. NS= Normal Saline, ALA= Alpha-Lipoic Acid

## DISCUSSION

Alpha lipoic acid showed beneficial effect in this our study after subjecting the mice to three TST tests at different stages of chronic mild stress test. Alpha lipoic acid at the dose 200 mg/kg reversed the effect of chronic mild stressors by significantly decreasing the immobility time (behavioural despair) at the last TST of the test. These our findings clearly depicted that ALA might possess antidepressant-like activity in the mice. This is in conformity with hypothesis of Salazar (2000) that ALA might possess antidepressant activity by increasing insulin sensitivity that can lead to increase in tryptophan absorption with subsequent increase in serotonin. Similarly, Lin et al. (2016) reported that ALA prevented endoplasmic reticulum stress-induced insulin resistance by enhancing mitochondrial functions. In addition, Natalia et al. (2009) found ALA possessing ability in increasing glutathione (GSH) that help in decreasing depression via redox dependent mechanisms of various cellular targets that decrease oxidative stress. However, Brian et al. (2013) did not find significant antidepressant effect of ALA at higher doses of 600-1800 mg/kg in combination of acetyl-L-carnitine (ALCAR) in depressed bipolar human patients.

The possible mechanisms via which ALA prevents depression might be via decreasing oxidative stress by increasing GSH level in the brain (Kate et al. 2009; Natalia et al. 2010) or reducing 8-hydroxy-2-deoxyguanosine and tissue damage mediated by heavy metals intoxication (Samy et al. 2014; Jolanta et al. 2015) or via reducing lipid peroxidation caused by MDA (Saraswathy et al. 2015; Jolanta et al. 2015). Another possible mechanism might be via reducing inflammation in line with cytokine hypothesis of depression (Maes 2008), ALA was found capable of increasing GSH that increases transcription anti-inflammatory factor 2 (Nrf2) with prevention of upregulation of TNF-alpha in brain endothelial cells (Kate et al. 2009) or suppressing expression of genes for TNF-alpha and interleukin-alpha (Jolanta et al. 2015). Another possible mechanism might be via reducing insulin resistance by increasing insulin sensitivity and tryptophan (Salazar 2000; Lin et al. 2016).

Alpha lipoic acid at dose of 200 mg/kg did not significantly affect motor activity of the mice in the open field test. This finding is in conformity with that of Saraswathy et al. (2015) who found that ALA at doses 50, 100 and 200 mg/kg did not significantly affect spontaneous motor activity of rats using Actophotometer. Our findings depicted that ALA might possibly possess antidepressant-like activity and lacks psychostimulant property. Cryan et al. (2005) reported that psychotonics are clinically ineffective as antidepressants. They show anti-immobility effects in the TST but increase locomotor activity. In order to detect the possibility of a false positive result in anti-immobility effects, locomotor activity test is highly useful.

Our findings revealed that ALA at dose 200 mg/kg did not significantly affect short term working memory (percentage preference) of the mice. However, Stoll et al. (1993) found that ALA at dose of 100 mg/kg improved long-term memory of aged female Naval Medical Research Institute (NMRI) mice but could not significantly affect that of young mice subjected to habituation of open field test after administration of the ALA for 15 days. In addition, Saraswathy et al. (2015) found that ALA (at doses 50, 100 and 200 mg/kg) reversed phenytoin-induced memory impairment of rats subjected to elevated plus maze test in a dose dependent fashion. Similarly, ALA reversed the cognitive deficit in high fat fed (HFF) rats in NORT (Manuel et al. 2016). Possibly, the ALA mechanism of ameliorating memory impairment might be via prevention of alterations in insulin signalling thereby improving memory via increasing vesicular glutamate transporter 1 (VGlut 1) which was found impaired in

frontal regions of HFF Alzheimer's rats (Manuel et al. 2016).

Altogether, the present results reveal ALA possesses antidepressant potential in mice and the anti-immobility observed in TST was not due to psychostimulation.

## Conflict of Interest

None declared.

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