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Potential effects of Alpha lipoic acid on behavioral alteration and glutamate accumulation during d-gal-induced brain aging in male rats

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Abstract--Background: Alpha lipoic acid has both hydrophilic and hydrophobic characteristics and is abundantly distributed in cellular membranes and the cytoplasm. It is among the top cell-protective antioxidants. Material and methods : The present work investigated the possible therapeutic effects of alpha lipoic acid in a male rat model of brain aging induced by D-galactose. Four equal-sized groups of 40 male rats were randomly assigned: G1, the control group, G2, and G3, which each received daily doses of 200 mg/kg of D-gal for 30 days. Alpha lipoic acid was given orally for 30 days to the G4 D-gal + alpha lipoic acid group at 200 mg/kg bw, IP. daily with 100 mg/kg. thirty days of IP. Glutamate is deposited in the brain, according to research on behavioral alterations and brain glutamate. Indicators of oxidative stress are increased Our Results show that whereas brain glutamate deposition declines in the D-gal model of aging, the Forced Swimming Test (FST) and Morris Water Maze Test considerably rise (MWM). According to the study's findings, D-gal brain damage can be enhanced by intubating 100 mg/kg B.W.IP of ALA to counteract its unfavorable effects. Results: value of tissue glutamate shows a significantly decrease ($p \leq 0.0001$) after four weeks in G2 treated group when compared to G1, G3 and G4, also there was no significant ($p \geq 0.0001$) change between G1, G3 and G4 groups. In addition, value of Forced Swim Test shows a significant decrease ($p \leq 0.0001$) in G2 group when compared with G1, G3 and G4 groups. On the other hand there is no significant ($p \geq 0.0001$) between G1, G3 and G4 groups. The

main value of Morris water maze Test (MWM) there was a significant increase ($p \leq 0.0001$) in G2 group when compared to G1, G3 and G4. Also, there was no significant ($p \geq 0.0001$) change between G1, G3 and G4 groups. Conclusion: Alpha Lipoic Acid improved neurodegeneration in a rat model of aging by D.Gal by attenuating ROS production, ER stress, mitochondrial dysfunction, excitotoxicity, and apoptosis.

Keywords--glutamate, D-galactose, alpha lipoic acid, the morris water maze test, the forced swimming test, brain aging, male rats.

Introduction

D-galactose (D-gal) has been considered an artificial aging model which induces oxidative stress and inflammatory response resulting in memory and synaptic dysfunction. (Kumar, A., Prakash 2011). Chronic systemic administration of D-gal in rodents has been extensively used as an animal model for brain aging in various anti-aging studies. (Haider, S. et al., (2015). It has been reported that animals receiving chronic successive administration of D-gal (50–500 mg/kg) for 4–8 weeks D galactose is a monosaccharide sugar that is about same sweet as glucose. The chimerical formation is $C_6H_{12}O_6$ D galactose is found in many foods such as milk, butter, cheese, yogurt, honey, beets, plums, cherries, figs, and celery (Azman & Zakaria, 2019). The maximal recommended daily dose of galactose for healthy adult is 1.5 g/kg/day (maximum 50 g/day), and excreted from the body within about 8 h after ingestion (Morava et al., 2014; Wong *et al.*, 2017) At high levels, it can be converted into aldose and hydroperoxide under the catalysis of galactose oxidase, resulting in the generation of reactive oxygen species (ROS) (Wu *et al.* 2008). The biological importance of galactose, however, goes beyond its importance as a nutrient and a metabolite (Bo-Htay *et al.*, 2018). Galactose is an omnipresent epimer of glucose that was first described by Louis Pasteur in 1856 following Erdmann's observation that hydrolysis of the milk sugar lactose yields a substance that is not glucose. Both in its free form and attached to other molecules forming oligo- or polysaccharides, all living organisms. (Coelho, A.I.; Berry, 2015). Aging is the process of becoming older, It is usually associated with dynamic changes in the biological, psychological, physiological, environmental, behavioural and social processes (Dras *et al.*, 2000). D-galactose causes aging changes related to natural aging processes, such as shorter lifespan, neurodegeneration and cognitive dysfunction, advanced end-product glycation (AGE) formation and oxidative stress, and transcriptional gene changes (Cui *et al.*, 2004; Cardoso et al., 2015; Tian *et al.*, 2019).

Alpha-lipoic acid (ALA), also known as 1, 2-dithiolane-3-pentanoic acid, is an endogenous essential cofactor in the mitochondrial complex that catalyzes the decarboxylation of pyruvate and alpha-ketoglutarate. Because of its antioxidant action, exogenous ALA is utilized as a dietary supplement in significant areas like Europe, the United States, and Japan (Takahashi H, Bungo Y, 2011). Additionally, it has been demonstrated in rats that ALA lessens oxidative stress damage, improves mitochondrial performance, and treats memory impairment by reversing the structural degeneration of the mitochondria that comes with aging

(Liu J, Head E, 2002). Multiple sclerosis and diabetic polyneuropathy are only a couple of the neurological disorders that are frequently treated using an antioxidant called alpha-lipoic acid (ALA). Through eating, it enters the body and crosses the blood-brain barrier (Biewenga GP, 1997). It is safe at therapeutic dosages (Shay KP, Moreau RF, 2009). This powerful antioxidant affects numerous physiological processes, including the direct scavenging of free radicals, recycling, metal chelation, endogenous antioxidant renewal, and modulation of transcription factor activity. It has been shown to improve endothelial function, blood flow, and glutathione synthesis, all of which are crucial for regulating the expression of several anti-inflammatory and antioxidant genes (Biewenga GP, 1997, Moini H, Packer L, 2022 -,; Guo S, Bragina O, 2008). Our aim here is to investigate the influence of alpha-lipoic acid in D. galactose-induced brain aging, this can be done firstly by evaluating of the behavioral analysis and memory by using Forced Swimming Test (FST) and Morris water maze test (MWM), respectively. Secondly, determining of glutamate- containing neural brain tissue.

Results

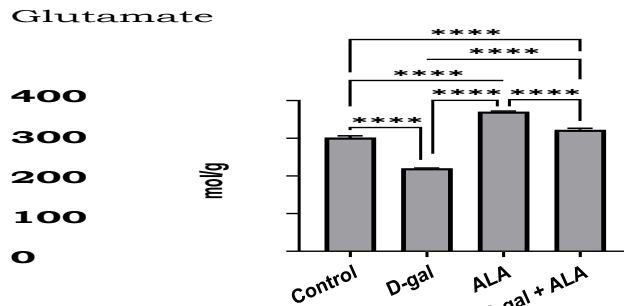


Figure 3.1. Glutamate level following D-gal and ALA in male rats

The level of glutamate was significantly decreased following D-gal to control group ($P < 0.0001$). ALA significantly increased glutamate level compare to control and D-gal groups ($P < 0.0001$). In addition, treated group with ALA + D-gal significantly increased glutamate level compare to control and D-gal groups ($P < 0.0001$). Data are expressed as mean \pm SEM, $n = 5$. The main value of tissue glutamate shows a significantly decrease ($P < 0.0001$) after four weeks in G2 treated group when compared to G1, G3 and G4, also there was no significant ($P < 0.0001$) change between G1, G3 and G4 groups. The main value of Glutamate was (301.8 \pm 0.62, 220.4 \pm 0.42, and 370.6 \pm 1.20, 322.2 \pm 0.78) for groups Control, D-gal, ALA and ALA + D-gal.

Forced Swimming Test

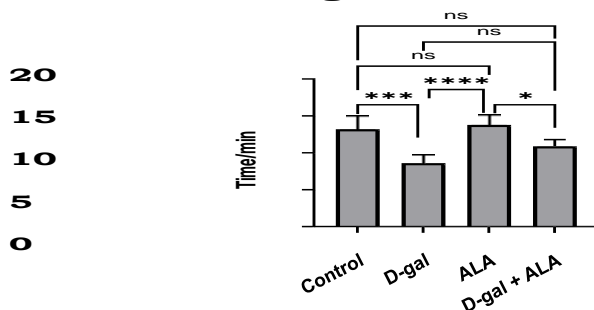


Figure 3.2. Forced swimming test following D.gal and ALA in male rats
The level of FST was significantly decreased following D-gal to control group ($P < 0.0001$). ALA significantly increased FST level compare to D-gal groups ($P < 0.0001$). In addition, treated group with ALA+ D-gal significantly increased FST level compare to D-gal groups ($P < 0.0001$). Data are expressed as mean \pm SEM, $n = 5$. The main value of Forced Swim Test shows a significant decrease ($p < 0.0001$) in G2 group when compared with G1, G3 and G4 groups. On the other hand there was no significant ($p \geq 0.0001$) between G1, G3 and G4 groups. The main value of Forced Swim Test was (13.2, 8.6, 13.8, and 10.8) for groups Control, D-gal, ALA and ALA+D-gal).

Morris water maze Test

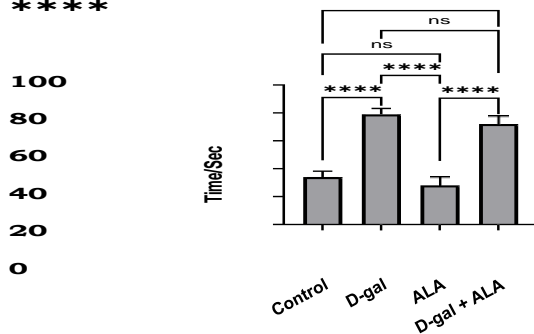


Figure 3.3. Morris Water Maze test following D.gal and ALA in male rats

The level of MWMT was significantly increased following D-gal to control group ($P < 0.0001$). ALA significantly decreased MWMT level compare to D-gal groups ($P < 0.0001$). In addition, treated group with ALA+ D-gal significantly decreased MWMT level compare to D-gal groups ($P < 0.0001$). Data are expressed as mean \pm SEM, $n = 5$. From The main value of Morris water maze Test (MWM) there is a significant increase ($p < 0.0001$) in G2 group when compared to G1, G3 and G4. also there was no significant ($p \geq 0.0001$) change between G1, G3 and G4 groups. The mean values at the end of the experiment were (34, 79, 28, and 72) for groups Control, D-gal, ALA and ALA+D-gal).

Materials and Methods

Protocol for experimentation

Forty (40) white male albino rats weighing 200–220g each were employed in the current investigation. Their ages ranged from 12 to 15 weeks, and they were acquired from the college of pharmacy at the University of Kerbala in Iraq. The rats were kept in well-maintained, specialized plastic cages with 12 hours of light per day and relative humidity levels of 50–5 percent. To give them time to acclimate to the typical experimental settings, they were kept for a period of two weeks. Forty (40) white albino rats were divided into four (4) groups of ten (10) each, and they were given the following care for four weeks. As a control, G2 rats of this group were given DMSO injections with 0.5 ml of normal saline, IP. administered D-galactose at a rate of 200mg/kg BWIP. (Li et al., 2015). The G3 rats in this group received an injection of 100mg/kg B.W.IP. of alpha lipoic acid (H. Jing, J. Li, J. Zhang et al., 2018). This group's G4 rats received 200mg/kg B.W.IP. of D-galactose and 100mg/kg B.W.IP. of alpha lipoic acid injections (Li et al., 2015).

Collected of the samples

To ensure that the tissue was spread evenly, weighed tissue samples were homogenized in 200 ul of 0.1 N perchloric acid for 150 seconds with squishers. The homogenates were then carefully separated from the supernatants by centrifuging them for 30 minutes at a low temperature (four degrees Celsius). At this stage, the supernatants can either be immediately removed for analysis or chi1. Determining the brain tissue's glutamate content.

Determining the ELISA method brain tissue's glutamate content

M. Nowicki, J. Kit Kwinta, and A. Kit Grabowska (2011).

Determination of Forced Swimming Test (FST)

When evaluating the rat, the method of (Cryan, J. F., Markou, A., 2002; Doron, R., et al. 2014).

Results of the Morris Water Maze Test (MWMT)

Rat size was determined by using (Morris, R.G.M., , 1993).

Discussion

Effect of D-Galactose, Alph Lipoic Acid and there combination on Glutamate in adult male rats

To comprehend the effects of aging and dementia on neurotransmitter levels and how that is related to insulin resistance, we looked at the effects of long-term D.gal administration on brain levels of acetylcholine and glutamate, which are closely linked to memory function and significantly decreased in AD patients. The

results showed that both neurotransmitter levels drastically decreased. When insulin transmission is disturbed, acetyl-CoA, a precursor to acetylcholine, is reduced, which lowers acetylcholine synthesis and impairs memory (Rivera EJ, Goldin A, 2005). Similar to how decreased glucose absorption results in lower levels of alpha ketoglutarate, a precursor to the excitatory amino acid glutamate, and subsequently lower levels of glutamate, in conditions of insulin resistance (Bordji K, Becerril-Ortega J, 2011). We've shown that giving ALA (100 mg/kg) right away after an ischemia injury had long-lasting (30 days) neurorestorative effects against the neuronal damage caused by cerebral infarction in rats. Additionally, these long-lasting neurorestorative effects of ALA might be brought about, at least in part, by enhanced neuroproliferation. Previous *in vivo* studies on the neuroprotective effects of ALA have only looked at how it lowers oxidative stress. Given the increased usage of ALA by the general population, the aforementioned findings may be important in the therapeutic environment. In several animal models, ALA treatment has been shown to reduce infarct size (Panigrahi M, Sadguna Y, (1996); Connell BJ, 2011). These earlier studies assessed the effects of ALA pretreatment, ranging from a single acute injection to many daily injections for up to 30 days, its efficiency in studies have been conducted on ALA (Panigrahi M, Sadguna Y) (1996).

Effect of D-Galactose, Alpha Lipoic Acid and there combination on Forced Swimming Test (FST) in adult male rats

According to this study (Allen PJ, D'Anci KE, 2010), ALA demonstrates a synergistic antidepressant influence and antidepressant-like response while delaying the beginning of depressive-like behavior. The FST is the most popular behavior test for antidepressant screening (Deussing JM.2006). Following initial attempts for escape, mice settle into their habitual motionless position in the impenetrable water-filled beaker. During the FST, immobility duration was recorded as a specific depressive-like phenotype, or behavior suggestive of despair. Prior to the FST, no appreciable difference was observed between rats treated with different medications and normal rats, therefore variations in the time spent immobile during the test may be interpreted as depressive-like behavior in animals. The interaction of medicine with brain mitochondria (Serkova NJ, Christians U, 2004). This analysis led to the following three main results, In the first case, intrahippocampal treatment of kainic acid was linked to abrupt spontaneous seizures, as well as interruptions in animal performance in the Morris water maze Test (MWM) and Forced Swimming Test (FST), as was evident by a lower alternation score. Secondly, reducing retention and recall abnormalities in the forced swimming test, enhancing short-term spatial memory performance in the labyrinth, and alleviating spontaneous convulsions by pretreating kainite rats with -LA at a dose of 100 mg/kg. Thirdly, one of the beneficial impacts ALA in this research may be the reduction of oxidative stress in the brain regions in charge of learning and memory, they are glutamate receptors (Ben-Ari, 1985; Ben-Ari & Cossart, 2000).

Effect of D-Galactose, Alph Lipoic Acid and there combination on Morris water maze Test (MWM) in adult male rats

Rats were aged and developed Alzheimer's disease (AD) associated with senility in the current investigation using D-gal. An experimental animal's entire body, including the brain, can age using this paradigm, exhibiting symptoms that are notably related to Alzheimer's disease (Hunter S, Arendt T, Brayne 2013; 30 Grieb P.2015). The current findings showed that long-term treatment of D-gal reduced the animals' average weight, a fact that could be explained by aging and muscle mass loss (Stefanova NA, 2014). In the activity cage test, there was no discernible difference in the animals' spontaneous locomotor behavior across all groups. In contrast, results from the MWM test showed that continuous D-gal treatment reduced the rats' capacity for memory and learning. These results are consistent of three key conclusions from this investigation as follows: Firstly, lower alternation scores in the first example indicated that intra-hippocampal administration of kainic acid was associated with sudden spontaneous seizures as well as disruptions in animal performance in the Morris water maze Test (MWM) and Forced Swimming Test (FST). Secondly, pretreatment of kainite rats with ALA at a dose of 100 mg/kg reduced retention and recall problems in the forced swimming test, improved short-term spatial memory performance in the labyrinth, and reduced spontaneous convulsions. Thirdly, decreasing oxidative stress in the parts of the brain responsible for learning and memory may be one of the positive effects of ALA in this research. They function as glutamate receptors (Ben-Ari, 1985; Ben-Ari & Cossart, 2000) with earlier findings reported in several literatures (Li H, Kang T, 2016; Rehman SU, 2016).

Conclusion

The results from this study indicate that Alph Lipoic Acid abrogated D-gal-induced hippocampal aging in rats and attenuating ROS production associated. In addition, Alph Lipoic Acid improved neurodegeneration by reducing ER-stress-mediated glutamate excitotoxicity, mitochondrial dysfunction, and apoptosis, and thereby improved short-term spatial learning and memory.

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