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Ameliorate role of alpha lipoic acid on the beta amyloid accumulation and some serum oxidant/antioxidants levels in brain aging induced by d-gal in male rats

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Abstract---Alpha lipoic acid has both hydrophilic and hydrophobic properties and is widely dispersed in cellular membranes and the cytoplasm. It is one of the most powerful antioxidants for cells. The current research looked at the possible therapeutic benefits of alpha lipoic acid) in a male rat model of D-galactose-induced brain aging. G1 normal as control group, G2 animals were given 200 mg/kg bw.IP. daily of D-gal group for 30 days, and G3 animals were given 100 mg/kg. G4 D-gal + alpha lipoic acid group 200mg/kg bw.IP. daily with 100 mg/kg. IP every day for 30 days. The accumulation of Beta amyloid in the brain and changes in serum oxidant/antioxidant levels. Increased levels of oxidative stress indicators our data demonstrate that the D-gal aging model causes an increase in brain Beta amyloid deposition, as well as a substantial rise in serum MDA, GSH, CAT, and SOD levels. To summarize, our research focused on the brain damage caused by D-gal, which can be mitigated by intubating 100 mg/kg B.W.IP. of ALA to counteract its negative effects.

Keywords---Alpha lipoic acid, beta amyloid, D-galactose, brain aging, male rats.

Introduction

D-galactose (D-gal) is a synthetic aging model that impairs memory and synaptic function by causing oxidative stress and an inflammatory response (Kumar, A., Prakash 2011). Chronic systemic injection of D-gal in rats has been widely used as an animal model for brain aging in several anti-aging studies (Haider, S. et al., 2003). (2015). Animals given a chronic dosage of D-gal (50–500 mg/kg) for 4–8 weeks had a sweet taste that was similar to glucose. D-gal is a monosaccharide sugar with a pleasant taste similar to glucose. The chimerical formation is $C_6H_{12}O_6$. D-gal may be found in a variety of foods, including milk, butter, cheese, yogurt, honey, beets, plums, cherries, figs, and celery (Azman & Zakaria 2019). The Leloir pathway metabolizes Dgal and is named after Luis Federico Leloir, who first described it (Frey, 1996; Walter & Fridovich-Keil, 2014). Because -D-gal is the pathway's active form, galactose mutarotase assists in the conversion of -D-gal to -D-gal in the first step. Galactokinase then converts -D-galactose to galactose 1-phosphate by phosphorylating it. In the third step, D-galactose-1-phosphate uridylyltransferase converts galactose 1-phosphate to UDP-galactose using UDP-glucose as the uridine diphosphate source. Finally, UDP-galactose 4-epimerase recycles UDP-galactose to UDP-glucose in the transferase phase. D-glucose 1-phosphate is also converted to D-glucose 6-phosphate by phosphoglucomutase. Fridovich-Keil and Walter (Walter & Fridovich-Keil, 2014). Alzheimer's disease (AD) is the most prevalent neurodegenerative disease that causes dementia in middle-aged and elderly people (Hager et al., 2001; Gonzalez et al., 2014; Irwin et al., 2016). Alzheimer's disease (AD) is characterized by the loss of cognitive functions such as memory, language, and reasoning over time (Di Domenico et al., 2015). LA's effects on experimental AD models have been investigated (Jesudason et al., 2005; Siedlak et al., 2009; Ahmed 2012; Sancheti et al., 2013).

ALA is a unique antioxidant that can rapidly quench free radicals, has an amphiphilic nature, and has no harmful side effects (Gora et al., 2011). ALA has anti-inflammatory effects as well. Another benefit of ALA is that it is soluble in both water and fat, allowing it to go to all regions of the body. It can access particular sections of the body due to its unique features.

Aim

Materials and Methods

The aim of current study is to investigate the amelioration effect of alpha-lipoic acid on the brain aging induced by D.galactose of the following.

1. estimation of the Brain tissue beta amyloid and serum oxidative and antioxidant.

The Experimental protocol

The rats utilized in this investigation were forty white male albino rats weighing 200–220g and ranged in age from 12 to 15 weeks. The animals were kept in special plastic cages and given the proper conditions and ventilation, including a 12-hour daily light cycle and a relative humidity of 50–50%. They were kept in the

lab for two weeks in order to adapt to the normal experimental settings. Forty white albino rats were randomly divided into four groups of 10 rats each for four weeks.

This group's G1 rats were injected with DMSO with normal saline (0.5) ml, IP, as a control., This group's G3 rats were given 100mg/kg B.W.IP. of Alpha lipoic acid. G4 rats in this group were given 100mg/kg B.W.IP. of Alpha lipoic acid and 200mg/kg B.W.IP. of D-galactose (Li et al., 2015).

Collect of the blood samples

A cardiac puncture was used to retrieve 5 mL of starving blood from the heart, and the blood samples were subsequently put in a test tube. The serum was separated by centrifugation at 3000 r/min for 5 minutes in a special gel tube without an anticoagulant, and the separated serum was deposited in Eppendorf's tubes and frozen at -20 ° C until the measurements were finished. Tissue samples were weighed and homogenized for 150 seconds in 0.1 N perchloric acid (200 l) (to guarantee tissue dispersion). The homogenates were centrifuged for 30 minutes at 4 degrees Celsius in a cold atmosphere, with the supernatants carefully collected. At this stage, the supernatants can be frozen (-80oC) or collected directly for examination.

1. Determination of brain tissue Beta amyloid.

brain tissue rat was measured following the method of ELISA Kit Grabowska, A.; Nowicki, M.; Kwinta, J. (2011).

2. Determination of Serum Malondialdehyde (MDA) concentration

Malondialdehyde was estimated by Thiobarbituric acid (TBA) assay method of Buege & Aust, 1978 on spectrophotometer

3. Determination of serum Reduced Glutathione concentration(mg/dl)

Reduced glutathione was measured following the method of Sedlak and Lindsay (1968),

4. Determination of serum catalase concentration:

Serum catalase concentration was measured following the method of (Hadwan and Abed. 2016) .

5. Determination of serum concentration of superoxide dismutase (SOD)

Serum superoxide dismutase was measured following the method of Marklund, S., & Marklund, G. (1974),

Results & Discussion

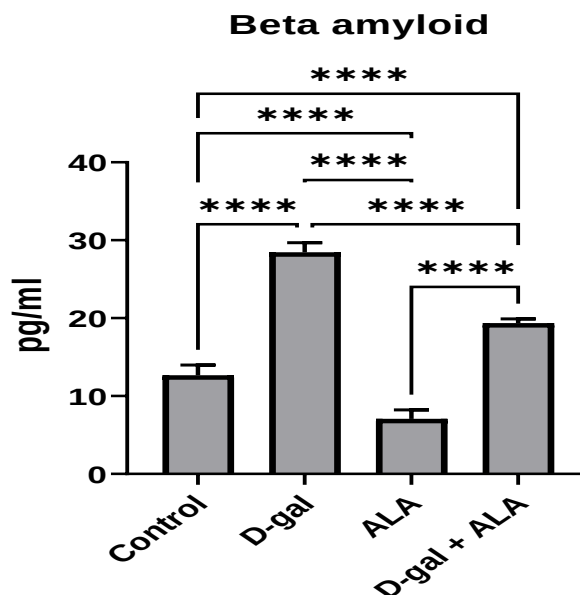


Figure 3.1. Beta amyloid (BA)level following D.gal and ALA in male rats.

BA levels were significantly greater in the D-gal group than in the control group ($P < 0.05$). ALA significantly lowered BA levels when compared to the control and D-gal groups ($P = 0.0001$). In addition, as compared to the control and D-gal groups, the ALA+ D-gal group exhibited significantly lower BA levels ($P = 0.0001$). The data is shown as a mean standard deviation of the mean ($n = 5$).

Figure 3.1 demonstrates that beta amyloid levels were considerably greater ($p < 0.0001$) in the D-gal injected group than in the other groups. this comprehension with (Hua et al.,2007). An increase in reactive oxygen species (ROS) produces a rise in Beta amyloid, which promotes brain aging and neuronal degeneration (Liu et al.,2020). The incomplete reduction of molecular oxygen yields reactive oxygen species, which are radicals and compounds (ROS). They are produced in small quantities during oxygen metabolism, as a result of four successive 1-electron reductions of O_2 that produce H_2O . They play an important role in cell homeostasis and signaling (Devasagayam et al.,2004). Free radicals are necessary for the formation of A. This has substantial ramifications since it implies that mitochondrial dysfunction has a role early in the etiology of sporadic AD. A may also promote mitochondrial malfunction and oxidative stress, possibly speeding up amyloid genic APP processing, because mitochondrial dysfunction linked with increased mitochondrial ROS generation is known to be integrally implicated in the aging process (Leuner et al.,2012). Because GSH reduces ROS in brain cells and oxidative stress in nerve cells, there was no significant difference in beta amyloid levels between the combination and D-gal groups ($p < 0.0001$). Beta amyloid levels in the GIII receiving ALA group were significantly lower than in the GI, GII, and GIV groups ($p < 0.0001$). The findings of this study are in line with those of other investigations (Yasser et al.,2015). ALA is found in

plant and animal tissues, as well as cellular membranes and the cytoplasm, and has both hydrophilic and hydrophobic characteristics (Deng et al., 2013). To have a positive impact on metabolic processes within the cell, improve cellular energy generation and minimize free radical creation. Panner selvam and colleagues (2002) Lipoic acid, as an antioxidant, quickly penetrates the blood-brain barrier and accumulates in a variety of brain locations, according to research. It reduces the amount of hydroxyl radical produced by the Fenton reaction by scavenging peroxide and superoxide radicals. ALA has the ability to scavenge a variety of free radicals in a number of situations. It's been revealed that it can help the body replenish various endogenous antioxidants (Bustamante et al., 1998). The role of ALA in the prevention of lipid peroxidation. It might be due to lipoic acid's ability to interfere with oxidation processes in both the lipid and aqueous compartments of the cell, as well as its bioactivity to directly react with oxidation (Packer Etc., 1997).

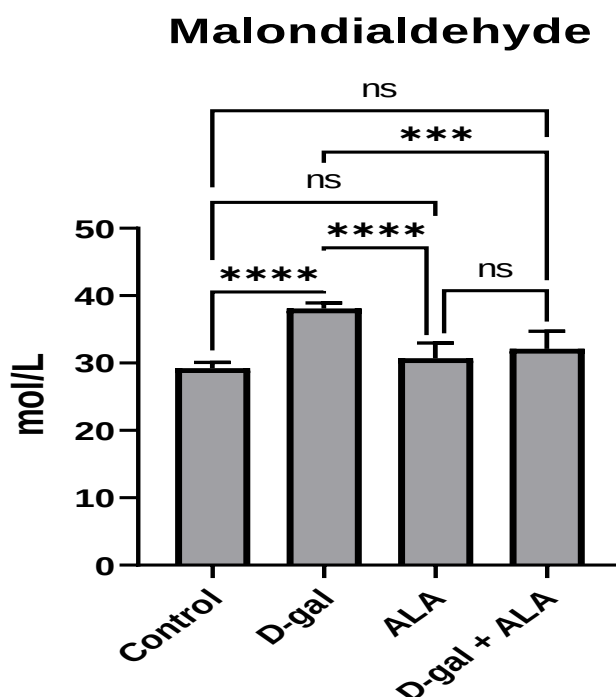


Figure 3.2. Malondialdehyde level following D.gal and ALA in male rats.

MDA levels in the D-gal group were significantly higher than in the control group ($P=0.0001$). ALA significantly lowered MDA levels when compared to D-gal groups ($P=0.0001$). Furthermore, when compared to D-gal, the ALA+ D-gal group exhibited significantly lower MDA levels. When the D-gal group was compared to the other groups (Figure 3.2), the results revealed a significant increase in MDA levels in the D-gal group (mean SEM, $n=5$). As a result of oxidative stress, D-gal raises serum MDA levels, as demonstrated by a significant increase in lipid peroxidation and a significant decline in GSH, SOD, and CAT. MDA is a lipid peroxidation byproduct produced by polyunsaturated fatty acid peroxidation in cells. An increase in free radicals causes MDA overproduction. (Kurutas., 2015).

Persistent IP with D-gal induces oxidative stress, which leads to an increase in lipid peroxidation as measured by blood MDA, according to the current study. MDA reduced considerably ($p < 0.0001$) in the combination group compared to the D-gal group.

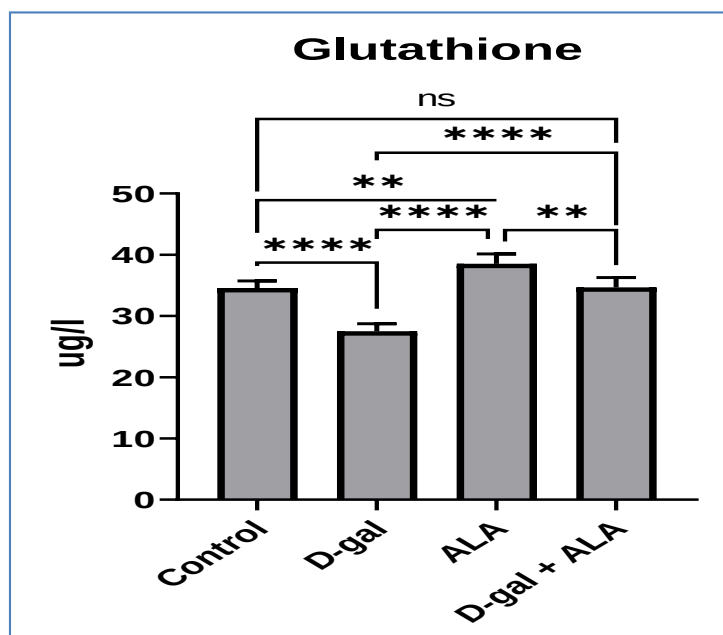


Figure 3.3. Glutathione (GSH) level following D.gal and ALA in male rats

GSH levels were considerably lower in the D-gal group compared to the control group ($P = 0.0001$). In comparison to the control and D-gal groups, ALA dramatically enhanced GSH levels ($P = 0.0001$). Furthermore, as compared to D-gal groups, the treated GSH levels were considerably higher in the ALA+ D-gal group ($P = 0.0001$). The data is shown as a mean standard deviation of the mean ($n = 5$). Glutathione (GSH) is an antioxidant produced by plants, animals, fungi, and certain bacteria and archaea. It can inhibit reactive oxygen species such free radicals, peroxides, lipid peroxides, and heavy metals from causing harm to critical cellular components. The blood GSH level in the D-gal group was significantly lower ($P < 0.0001$) than in the other groups. Increased ROS generation in the body after injection of 200 mg/kg.bw of D-gal for 4 weeks might be explained by D-gal raising oxidative stress and boosting ROS production, as well as limiting cysteine uptake and synthesis, resulting in a reduction in GSH and an increase in ROS. (Dröge, 2000). (2005). The combo group had a substantial increase ($P < 0.0001$) in serum decreased GSH when compared to the D-gal group. GSH is a critical component in mitochondrial energy metabolism, where it aids in the detoxification of lipid hydroperoxides and electrophiles as well as the defense against respiration-induced reactive oxygen species (Ribas et al., 2014). Furthermore, because mitochondria play such an important role in cell death activation and mode, mitochondrial GSH has been shown to regulate the level of sensitization to secondary hits that cause mitochondrial membrane permeabilization and the release of proteins trapped in the intermembrane space,

which then engage the molecular machinery of cell death once in the cytosol (Ribas et al.,2014).The acceleration of aging may be due to osmotic and oxidative stress.

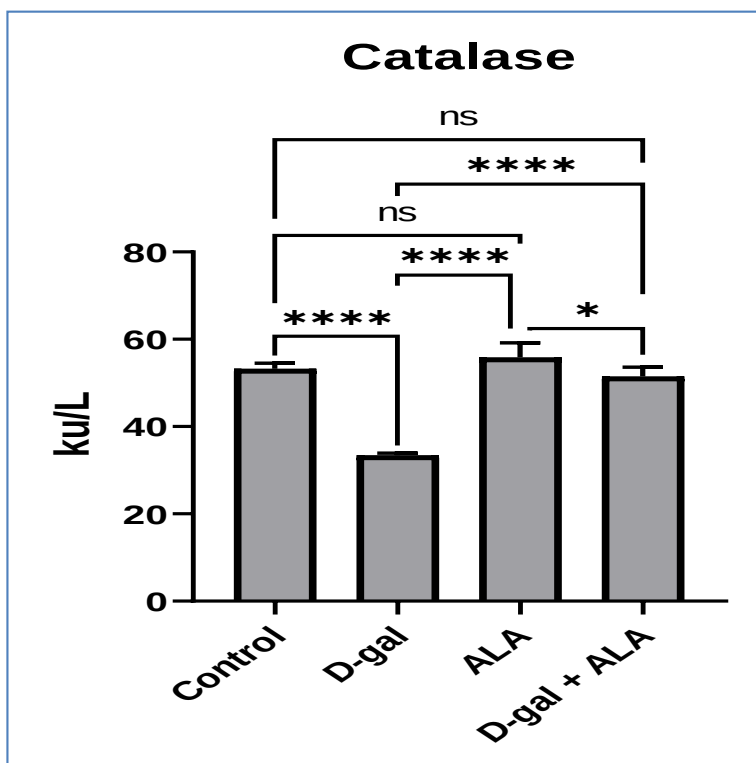


Figure 3.4.Catalase (CAT) level following D.gal and ALA in male rats

The level of CAT in the D-gal group was significantly lower than in the control group ($P=0.0001$). ALA significantly increased CAT levels ($P=0.0001$) as compared to D-gal groups. In addition, as compared to D-gal groups, the ALA+ D-gal group significantly increased CAT levels ($P=0.0001$). The data is shown as a mean standard deviation of the mean ($n=5$).

As a result of this process, hydrogen peroxide is created, which can be decreased further by catalase activity (CAT). This enzyme, along with glutathione reductase, is a member of the glutathione cycle (GR). However, the link between antioxidant level, lipid peroxidation, and aging appears to differ depending on the species, strain, sex, and organs studied in the experimental animals.. Epidermal cells that were vulnerable to superoxide and hydrogen peroxide produced more SOD, but cells that produced more CAT were shielded from the oxidants' effects. MDA levels were considerably lower ($p0.0001$) in the GIII ALA group than in the GI, GII, and GIV groups. The findings of this study are in line with those of other investigations (Yasser et al.,2015). ALA is found in plant and animal tissues, as well as cellular membranes and the cytoplasm, and has both hydrophilic and hydrophobic characteristics (Deng et al., 2013). It involves introducing a naturally occurring chemical into the body in order to have a positive influence

on metabolic activities within the cell, such as increasing cellular energy generation and lowering free radical creation. As an antioxidant, lipoic acid passes the blood-brain barrier and accumulates in the brain areas (Pannerseelvam et al., 2002.). It reduces the amount of hydroxyl radical produced by the Fenton reaction by scavenging peroxide and superoxide radicals. ALA has the ability to scavenge a variety of free radicals in a number of situations. It's been revealed that it can help the body replenish various endogenous antioxidants (Bustamante et al., 1998) The role of ALA in the prevention of lipid peroxidation. It might be linked to lipoic acid's ability to interfere with oxidation processes in both the lipid and aqueous compartments of the cell, as well as its bioactivity to directly react with oxidation (Packer et al., 1997). (Wonderful+ paraphrase) GSH, SOD, and CAT are all examples of antioxidants. Blood levels of (GSH,SOD,CAT) were considerably lower ($p < 0.0001$) in GII who received D.gal than in the control group. This result is in line with (Ali et al., 2016). In research, it was discovered that the pool of (GSH,SOD,CAT) was reduced as a result of cytotoxicity and oxidative stress (Wongcharoen et al., 2012). The findings of the study corroborate those of ALA supplementation, which showed improvements in total antioxidant capacity GSH, SOD, and CAT, as well as a decrease in MDA (Chirli, 2008 and Golbidi et al., 2011).. ALA is a natural chemical that may be produced by lipoic acid synthase in the mitochondria and absorbed from meals. It's an important part of mitochondrial energy metabolism. It is easily absorbed and dispersed throughout the body's tissues since it is both hydrophilic and hydrophobic. ALA's antioxidant activity is demonstrated in part by GSH activity. (Sarper et al., 2018) Another study discovered that ALA aids in the reduction of age-related changes in GSH levels, making exogenous GSH delivery to tissues such as the brain and heart impossible. (Suh and coworkers, 2004.)

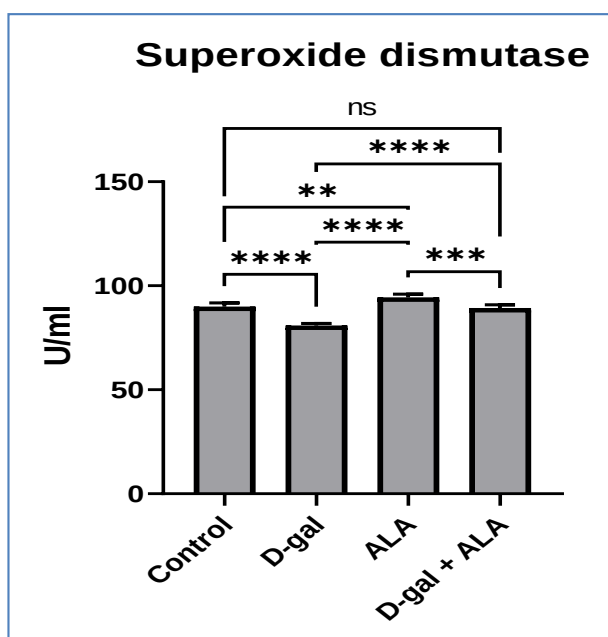


Figure 3.5. Superoxide dismutase (SOD) level following D.gal and ALA in male rats

The D-gal group had significantly lower SOD levels than the control group ($P=0.0001$). ALA significantly increased SOD levels as compared to D-gal groups ($P=0.0001$). In addition, as compared to D-gal groups, the ALA+ D-gal group dramatically increased SOD levels ($P=0.0001$). The data is provided as a mean SEM ($n=5$). When comparing the D-gal injection group to the other groups, there was a significant decline ($P=0.0001$) in CAT& SOD levels from (3-5). Galactose oxidase produces D-galacto-hexodialdose and hydrogen peroxide from excess D-gal, whereas aldose reductase transforms it to galactitol. These chemicals can accumulate in cells, causing. SOD aids in the prevention and treatment of diseases caused by free radicals like superoxide. When there is an overabundance of superoxide anion radicals, or the SOD concentration is low, oxidation occurs (Younus, H.) (2018). cells, as well as mitochondria and the cytoplasm (Selvaratnam & Robaire, 2016). During normal oxidative respiration, organisms continuously create ROS. Because it's a highly active chemical, it possesses unpaired electrons. SOD is an enzyme system capable of removing reactive oxygen species (ROS). SOD primarily distorts O_2 into H_2O_2 as an opening line of defense against ROS. (Pawlak et al., 1998) (Ghosh et al., 1998) (Pawlak et al., 1998) (Pawlak et al., 1998) (Pawlak et al., 1998) (2018). Superoxide anions are removed from the body by SOD.

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