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Role of alpha lipoic acid on some biochemical parameters and behaviorals of D-galactose induced neurological damage in male rats

Thesis

Submitted to the Council of the College of Veterinary Medicine, University Of Karbala in Partial Fulfillment of the Requirements for the Master of Degree of Science in Veterinary Medicine / Physiology

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بسم الله الرحمن الرحيم

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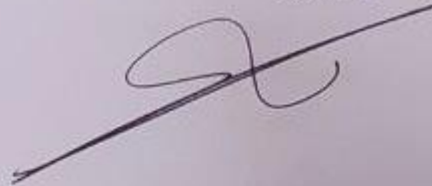

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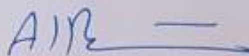

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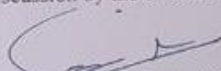

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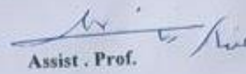

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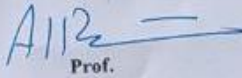

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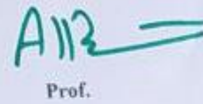

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Declaration

I hereby declare that this dissertation is my original work except for equations and citations which have been fully acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University of Kerbala or other institutions.

Haider Ali Yousif

/ / 2022

Dedication

If dedication is part of loyalty, I dedicate this research

To the one who paved the path of knowledge for me, to the one whose name I carry with pride... my dear father. To whom her supplication was the secret of my success and the most precious thing I possessed. To the symbol of love and the sea of tenderness...my mother

Sweetie to those who are my consolation and support in life... my brothers & sisters

*Thank you very much to my family (**my father * my mother * my wife * my brothers * my childrens**)*

*Thank you very much to my friends (**Dr. Wahb * Dr. Hussein * Dr. Ahmed * Dr. Amjad * Dr. Ithmar * Dr. Sadiq)***

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Haider Ali Yousif...

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List of Abbreviations

Abbreviations	**Meaning**
A.D	Alzheimer’s disease
AChE	Acetylcholinesterase

AGEs	advanced glycation end products
AGEs	Advanced glycation end products
AICD	APP IntraCellular Domain
ALA	Alpha Lipoic Acid
B.W	body weight
CAT	Catalase
CTF-β	C-terminal fragment-β
CVD	cardiovascular disease
D-gal	D-Galactose
DHLA	Dihydrolipoic acid
DLT	Dark &Light Test
ELISA	Enzyme-Linked Immunosorbent Assay
ENOS	Endothelial nitric oxide synthase
EPT	Elevated Plus Test
FST	Forced Swim Test
GSH	Glutathione
IP	Intraperitoneal injection
Kg	Kilogram
MDA	Malondialdehyde
Mg	Milligram
Mg/dl	milligrams per deciliter
Mg/kg	Milligram per kilogram
MWT	Morris Water Test
Ng/ml	Nanograms per milliliter
Nm	Nanometer
NP-SH	Non-protein sulphhydryls
OS	Oxidative stress

Pg/ml	Picograms per milliliter
r/min	Revolutions per minute
ROS	reactive oxygen spices
sAPP- β	Soluble *APP- β*
SOD	Superoxide dismutase
UDP	Uridine diphosphate
βA	beta-amyloid

Abstract

This study is carried out to investigate the protective role of alpha lipoic acid (ALA) on the central nerves system damage induced by d-galactose overload in male rats.

Forty adult male rats were divided into four groups (10 / group), the first group was injected with normal Saline for thirty (30) days and served as control group (control). Rats in the second group were injected with 200 mg/kg B .W IP for 30 days of (D-gal), rats of the third group (ALA) were injected with (D-gal) (IP) with 100 mg/kg B .W for 30 days, and the fourth group (D-gal+ALA) was injected D-gal 200 mg/kg B.W with ALA 100 mg/kg B.W. daily for 30 days. The experiment was carried out in the laboratories of the college of veterinary medicine, university of karbala.

Fasting blood samples were collected by cardiac punctures technique at thirty (30) days of experiments for collecting the brain tissue for measuring: beta amyloid, glutamate, serum acetylcholine Esterase(AChE) activity ,total anti-oxidant capacity(TAC), Malondialdehyde (MDA), reduced Glutathione(GSH) concentration, catalase (CAT), superoxide dismutase (SOD) activities and assessment of behavioral analysis forced swimming test (FST),light/dark test(LDT) and memory tests morris water maze Test(MWM),elevated plus-maze test (EPM).

The results showed a significant increase ($P \leq 0.05$) in the brain tissue beta amyloid value in D-gal treated group as compared to control, ALA and (D-gal+ALA) groups. There was a significant decrease ($P \leq 0.05$) in the tissue glutamate concentration in ALA treated group when compared to control, ALA and (D-gal+ALA). While, there were no significant ($P \geq 0.05$) changes between control, ALA and (D-gal+ALA) groups. In addition, the result showed a significant decrease in the serum (TAC) concentration ($P \leq 0.05$) in the d-gal treated group when compared to control , ALA and (D-gal+ALA), While there was no significant ($P \geq 0.05$) changes between control , ALA and (D-gal+ALA) groups. serum GSH concentration shows a significant decrease ($P \leq 0.05$) in D-gal group when compared with control, ALA and (D-gal+ALA) groups.There was no significant ($P \geq 0.05$) between control, ALA and (D-gal+ALA) groups.

The current study showed a significant increase MDA concentration ($P \leq 0.05$) in D-gal group when compared with control, ALA and (D-gal+ALA) groups.There was no significant ($P \geq 0.05$) D-gal change between control, ALA and (D-gal+ALA) groups, While, serum (CAT) activity showed a significant decrease ($P \leq 0.05$) D-gal when compared with control, ALA and (D-gal+ALA) groups. There was no significant ALA group ($P \geq 0.05$) change between control , ALA and (D-gal+ALA) groups .serum (SOD) concentration showed a significant decrease ($P \leq 0.05$) in D-gal group when compared with control , ALA and (D-gal+ALA) groups.There were no significant ($P \geq 0.0001$) d-gal changes between control, ALA and (D-gal+ALA).

Behavioral analysis and memory test showed a significant decrease in the FST ($p \leq 0.05$) in D-gal group when compared with control, ALA and (D-gal+ALA) groups.There was no significant ($p \geq 0.05$) D-gal between control, ALA and (D-gal+ALA) groups. While showed a significant ($p \leq 0.05$) increase in the (MWM) in D-gal group when compared to control, ALA and (D-gal+ALA), and there were no significant ($p \geq 0.05$) changes in the D-gal between control, ALA and (D-gal+ALA) groups.light room test showed a significant increase ($P \leq 0.05$) in D-gal treated group when compared to control, ALA and (D-gal+ALA), also there was no significant ($P \geq 0.05$) d-gal change between control, ALA and (D-gal+ALA) groups.Dark Room test shows a significant decrease ($P \leq 0.05$) in d-gal treated group when compared to control, ALA and (D-gal+ALA), also there were no significant ($P \geq 0.05$) D-gal changes between control, ALA and (D-gal+ALA) groups.

Elevated plus-maze test \ open arm showed a significant increase ($P \leq 0.05$) after 30 days in D-gal treated group when compared to control, ALA and (D-gal+ALA), also there were no significant ($P \geq 0.05$) D-gal changes between control, ALA and (D-gal+ALA) groups. Elevated plus-maze test \ closed arm showed a significant decrease ($P \leq 0.0001$) in D-gal treated group when compared to control, ALA and (D-gal+ALA), also there were no significant ($P \geq 0.05$) D-gal changes between control, ALA and (D-gal+ALA) groups.

In conclusion, our results revealed that ameliorated role of the ALA on the oxidative stress and deterioration of the behavior and memory in the male rats was induced by D-gal.

Chapter One: Introduction

Introduction

D-galactose (D-gal) has been considered as an artificial aging model which induces oxidative stress and inflammatory response resulting in memory and synaptic dysfunction (Conte *et al.,* 2021). Chronic systemic administration of D-gal in rodents has been extensively used as an animal model for brain aging in various anti-aging study (Haider *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-gal is a monosaccharide sugar that is about same sweet as glucose the chemical formation is $C_6H_{12}O_6$ D-gal 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) it 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 (BoHtay *et al*., 2018).Furthermore, together with glucose (in the form of disaccharide lactose) galactose is a cornerstone of animal milk that provides structural and metabolic support during the most sensitive developmental period (Coelho *et al*., 2015).Regardless of its importance as a nutrient and pertinent physiological role best reflected in glycolipids, or glycoproteins, galactose is ubiquitous in biological consequences of inherited defects of its metabolism, the biochemistry of galactose and its implications in health and disease remain enigmatic (Conte *et al*., 2021).

Galactose is extensively used for modeling aging-related pathophysiological processes in rodents (Azman & Zakaria, 2019).Oxidative stress (OS) has been proposed as the main driver mediating galactose-induced senescence, although exact pathophysiological mechanisms mediating detrimental effects are yet to be elucidated (Azman & Zakaria, 2019).D-gal is a physiological nutrient that is chemically a reducing sugar (Chen *et al*., 2006). It is found in abundance in milk, vegetables and fruits (Gropper, S. *et al*., 2000).D-gal interacts with various free amines in the protein architecture via non-enzymatic glycation, resulting in the generation of advanced glycation products this results in the generation of reactive oxygen species (ROS) also, excessive levels of D-gal in the body (either due to increased intake or decreased me-

tabolism) may lead to the formation of various ROS, chiefly through oxidative metabolism, and additionally through the secondary glycation pathway (Parameshwaran *et al*., 2010).ROS and advanced glycation products have been implicated in several age-related diseases like alzheimer's disease, diabetes mellitus, cancers and amyotrophic lateral sclerosis,etc (Budni *et al*., 2016).

Since d-gal induces the generation of these advanced glycation products and ROS, it is commonly employed as a model to enhance aging in rodents, thereby finding a place in rodent models for alzheimer's disease and other memory impairment disorders most of the studies that are currently available in the accessible literature mention that D-gal can successfully be used as a rodent model similarly, the temporal associations of these effects with the administration of D-gal are unclear While most authors have mentioned that chronic administration of D-gal can lead to impairment of learning and memory in rodents, the acute effects of the sugar are not well established a few studies have shown that this is not a standard model, as these changes produced are not consistent across all species and ages (Cardoso *et al*., 2015) .

(ALA) is a specific antioxidant; it can easily quench radicals, has an amphiphilic character, and does not exhibit any serious side effects (Gora *et al*., 2011). ALA also demonstrates anti-inflammatory properties as it is soluble both in water and in fat, which allows it to travel to all parts of the body because of its special properties, it is able to enter certain parts of the cell that most other antioxidants are not able to reach this compound acts by many mechanisms and can therefore be a very effective antioxidant hence, ALA is used in various diseases concerning age-dependent oxidative stress,it can be particularly effective in cardiovascular diseases, including ischemic heart disease, hypertension, heart failure, and atherosclerosis, where it may slow aging and prolong lifespan many studies have confirmed that ALA can improve vascular function and decrease the atherosclerotic plaque burden (Wollin & Jones, 2003).

ALA inhibits the vascular overproduction of endothelin I, the main vasoconstrictor (Takaoka *et al*., 2001).Furthermore, ALA significantly increases the synthesis of NO^-, the main vasodilator; it may also improve the redox state of the plasma and improve endothelium-dependent NO^- mediated vasodilation in addition, ALA ameliorates the loss of ENOS phosphorylation, which contributes to improve endothelial Function ALA act as a defensive agent versus risk factors of cardiovascular disease and may influence the CVD risk *via* the beneficial actions on LDL oxidation, blood

lipid profiles, plaque formation and hypertension (Wollin &Jones, 2003). ALA has the ability to scavenge ROS, metal chelating, and regenerate, glutathione, vitamins E and C (Singh &Jialal, 2008). ALA also has anti-inflammatory properties and can mend vascular function and decrease the atherosclerotic plaque burden dihydrolipoic acid (DHLA) the reduced form of ALA, is capable of exerting an antioxidant effect directly by donating electrons to a pro-oxidant or an oxidized molecule it can regenerate ascorbic acid from dehydroascorbic acid, and it can indirectly regenerate vitamin E back from its oxidized state as well, ALA metabolites have been shown to have antioxidant effects (Kwiecien *et al*., 2013).

ALA the oxidized form of dHLA, can exert an antioxidant effect but this does not mean there is any donation of electrons by ALA to a pro-oxidant or oxidized molecule, since there are none to give however, it has been documented that ALA can inactivate free radicals, which is a significant antioxidant effect (Packer *et al*., 2001). Also, the ability of ALA to chelate metals can produce an antioxidant effect dHLA can exert a pro-oxidant effect of donating its electrons for the reduction of iron, which can then break down peroxide to the prooxidant hydroxyl radical *via* the Fenton reaction thus, ALA and its reduced form dHLA, can promote antioxidant properties ALA has been effectively chelate toxic metals directly, and it also indirectly strongly supports the chelation of metals by its ability to increase glutathione levels inside the cells (Ghibu *et al*., 2009).

Aims of the study.

The overall aims of this research project are to investigate the amelioration effect of ALA on the brain aging induced by D-gal of the following.

1) Estimation of the biochemical and oxidative and antioxidant.

2) Neural brain tissue of glutamate and beta amyloid.

Chapter Two: Review of the Related Literature

2. Review of the Related Literature

2.1. D-galactose

2.1.1 D-galactose chemical properties

(D-gal) has been considered an artificial aging model which induces oxidative stress and inflammatory response resulting in memory and synaptic dysfunction (Conte *et al.,* 2021).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 *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-gal is a monosaccharide sugar that is about same sweet as glucose the chemical formation is $C_6H_{12}O_6$ D-gal is found in many foods such as milk, butter, cheese, yogurt, honey, beets, plums, cherries, figs, and celery (Azman & Zakaria, 2019).The term D come when the hydroxyl group on carbon 5 is located on the right side of the Fischer projection, as show in **Figure (2.1)**

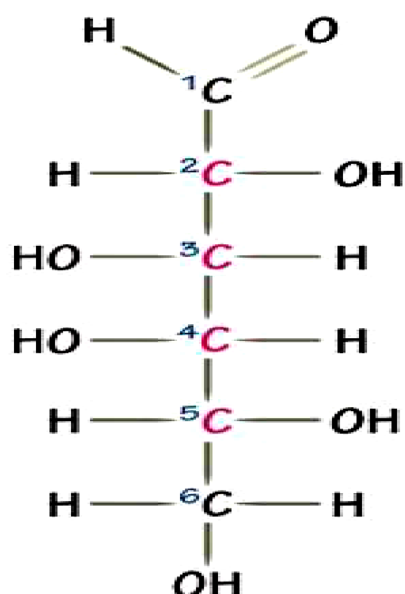

Figure 2.1: Chemical structure of D-galactose (Zaitoun *et al.,* 2018).

The maximal recommended daily dose of galactose for healthy adult is 1.5 g/kg/day (maximum 50 g/day), It can be 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 (BoHtay *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 *et al*., 2015).Furthermore, together with glucose (in the form of disaccharide lactose) galactose is a cornerstone of animal milk that provides structural and metabolic support during the most sensitive developmental period (Coelho *et al*., 2015).Regardless of its importance as a nutrient and pertinent physiological role best reflected in glycolipids, or glycoproteins, galactose is ubiquitous in biological consequences of inherited defects of its metabolism, the biochemistry of galactose and its implications in health and disease remain enigmatic (Conte *et al*., 2021).

Galactose is extensively used for modeling aging-related pathophysiological processes in rodents (Azman & Zakaria, 2019).Oxidative stress (OS) has been proposed as the main driver mediating galactose induced senescence, although exact pathophysiological mechanisms mediating detrimental effects (Azman & Zakaria, 2019).

2.1.2 D-galactose metabolism:

D-gal metabolized by the leloir pathway which named after luis federico leloir, who first described it (Walter & Fridovich-Keil, 2014). In the first step, galactose mutarotase facilitates the conversion of β-D-gal to α-D-gal since this is the active form in the pathway next, α-D-gal is phosphorylated by galactokinase to galactose 1-phosphate ,in the third step, D-gal-1-phosphate uridylyltransferase converts galactose 1-phosphate to UDP-galactose using UDP-glucose as the uridine diphosphate source finally UDP-galactose 4-epimerase recycles the UDP-galactose to UDP-glucose for the transferase reaction. Additionally, phosphoglucomutase converts the D-glucose 1-phosphate to D-glucose 6-phosphate (Walter & Fridovich-Keil, 2014).

2.1.3 Role of effect D-galactose Oxidative stress:

Oxidative stress is regarded as an imbalance between the oxidative and antioxidative reactions (Yahata & Hamaoka, 2016).Under normal physiological circumstance,reactive oxygen species (ROS) are maintained homeostasis and excessive ROS are eliminated by the antioxidant system including non-enzymatic components and antioxidant enzymes, such as glutathione (GSH), catalase (CAT), and superoxide

dismutase (SOD) (Jiang *et al*., 2016).However, the imbalance between the pro-oxidant and antioxidant activity will lead to the excess production of ROS, which can cause oxidative stress (Jiang *et al*., 2016).High level of D-gal can be oxidized by galactose oxidase to yield hydrogen peroxide, increased hydrogen peroxide causes a decrease in antioxidant enzymes like SOD furthermore, D-gal can initiate non-enzymatic glycation reactions to form advanced glycation end products (AGEs) after weeks or months, When AGEs react with their receptors (RAGE), ROS production occurs through NADPH oxidase activation H2O2, hydrogen peroxide; SOD, superoxide dismutase; RAGE, receptor for advanced glycation end products; ROS, reactive oxygen species (Wong *et al*., 2017).

2.1.4 Role of D-galactose on Aging

Aging is the process of becoming older and it is usually associated with dynamic changes in the biological, psychological, physiological, environmental, behavioural and social processes (Dras *et al*., 2000).D-gal causes aging changes related to natural aging processes, such as shorter lifespan, neurodegeneration and cognitive dysfunction, (AGE) formation and oxidative stress, and transcriptional gene changes (Cardoso *et al.,* 2015; Tian *et al.,* 2019).

Accumulating evidence suggests that mitochondrial dysfunction and oxidative stress play major roles in aging chronic administration of D-gal has been reported to cause deterioration of cognitive and motor skills that are similar to symptoms of aging and, therefore, is regarded as a model of accelerated aging because enhancing endogenous antioxidants is now widely regarded as an attractive therapy for conditions associated with mitochondrial oxidative stress (Parameshwaran *et al.,* 2010; Lian *et al*., 2017).D-gal is used to hasten the aging process in various tissues in rodent models and it has been shown to successfully mimic the oxidative alterations that take place in the natural aging process in various tissues (Yanar *et al.*, 2019).

2.2. Glutamate:

Glutamate is the most abundant excitatory neurotransmitter in the central nervous system (CNS) of mammals, its actions mediated by the activation of glutamate receptors these receptors participate in normal synaptic transmission, plasticity, synaptogenesis and neuronal maturation, and errant functioning of this system may

provoke some types of epilepsy or contribute to other different CNS disorders (Traynelis *et al*., 2010; Flores *et al*., 2012).

Glu receptors are classically divided into two large families: ionotropic and metabotropic the ionotropic glutamate receptors (iGluRs) participate in rapid neuro-transmission and they are classified into three types depending on the agonist that ac-tivates them with highest affinity: N-methyl-d-aspartic acid (NMDA) receptors (NMDARs); α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) recep-tors (AMPARs); and kainate (KA) receptors (KARs) these receptors form a channel with different selectivity depending on their subunit composition, all of them are permeable to Na+ and K+ Moreover, NMDARs are permeable to Ca2+, with some AMPARs and KARs also displaying Ca2+ permeability depending on their subunit composition (Traynelis *et al*., 2010).Metabotropic glutamate receptors (mGluRs) par-ticipate in "slow" neurotransmission, they are coupled to G-proteins, and they are di-vided into eight types (mGluR 1–8) and three groups: group I that includes mGluR1 and mGluR5 receptors positively coupled to phospholipase C (PLC); Group II that includes mGluR2 and mGluR3 receptors; and group III that includes mGluR4, mGluR6, mGluR7 and mGluR8 receptors, all negatively coupled to adenylate cyclase-mediated cAMP formation (Niswender &Conn, 2010).

The distinction between "ionotropic" and "metabotropic" receptors seems to be more complex than anticipated, and the three glutamate receptors typically consid-ered to be ionotropic (NMDARs, AMPARs and KARs) have also been defined as having additional non-ionotropic or direct metabotropic actions AMPARs are well known mediators of the majority of fast excitatory neurotransmission in the CNS, yet some of their actions are sensitive to G proteins. For instance, they activate MAPK kinases in a pertussis toxin (PTX) sensitive manner, suggesting the involvement of a G protein (Wang &Durkin, 1995).They interact with GI proteins (Wang *et al.,* 1997). Synaptically, AMPARs with a metabotropic role in the cerebellum have been found to participate in the control of GABA release onto Purkinje cells (Satake et al., 2004). NMDARs with a metabotropic action have been proposed to participate in long-term depression (LTD) in the hippocampus (Nabavi *et al*., 2013, but see Babiec *et al*., 2014) and cerebellum (Kakegawa *et al*., 2007).A metabotropic role for presynaptic NMDARs was defined in the visual cortex, controlling spontaneous (but not evoked) glutamate release (Abrahamsson *et al*., 2017; Bouvier *et al*., 2018).

Glutamate is the predominant excitatory neurotransmitter in the mammalian CNS. The neurotransmitter pool of glutamate is stored in synaptic vesicles and, upon depolarization, is released into the synaptic cleft in a Ca(2+)-dependent fashion. Glutamate is cleared from the synaptic cleft by high-affinity, Na(+)-dependent uptake carriers located in both neurons and glia. Glutamate acts on several distinct families of receptors, each of which has multiple subtypes with distinct pharmacologic and physiologic properties under some conditions, glutamate and related compounds act as excitotoxins and might participate in the events leading to neuronal damage and death in a variety of acute and chronic neurologic disorders the potential for glutamate to become an excitotoxin is highly dependent upon neuronal metabolic status. A great deal of interest in developing selective, well-tolerated glutamate receptor antagonists for the treatment of a variety of neurologic disorders exists (Jane et al., 2009).

The first glutamate receptors typically considered ionotropic to be attributed a physiological metabotropic activity were the KARs in the hippocampus (Rodríguez-Moreno &Lerma, 1998).KARs are typically tetramers made up of different combinations of GluK1-GluK5 subunits encoded by the *Grik* 1–5 genes GluK1-GluK3 may form homomeric or heteromeric receptors, while GluK4 and GluK5 may only participate in functional receptors when associated with any of the GluK1-GluK3 subunits. KARs have been described in different invertebrates like nematodes and flies (Lee *et al.,* 2002).There was mammals, In mammals, KARs have been found in virtually the entire nervous system, although their subcellular location has yet to be fully defined (Paternain *et al*., 2000; Huettner, 2003; Jane *et al*., 2009).Genetic deficits in *Grik* 1–5 genes or incorrect KAR activity are involved in some brain alterations and diseases, including epilepsy, ischemia, stress and anxiety, Autism Spectrum Disorders (ASDs), Schizophrenia, bipolar disorder and mental retardation (Lerma &Marques, 2013). **Figure (2.2)**

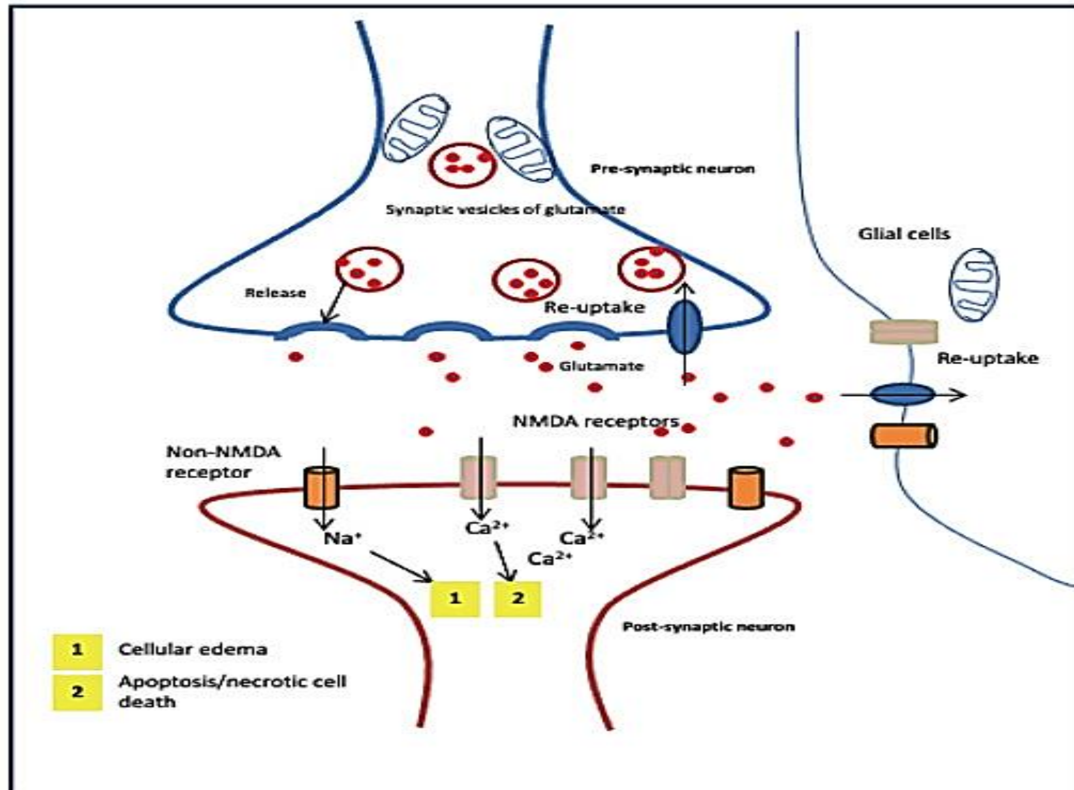

Figure 2.2: Mechanisms of glutamate excitotoxicity in the neuron glial unit. Glu is released from the presynaptic terminal of neuronal axons into the synaptic cleft and acts as a neurotransmitter the reuptake of extracellular glutamate occurs at the presynaptic terminals and adjacent glial cells Mitochondria provide energy for the reuptake of glutamate the excess binding of glutamate to NMDA receptors allows entrance of Ca^{2+} into the postsynaptic neuron, resulting into necrotic cell death/apoptosis, while the excessive glutamate binding to non-NMDA receptors allowing Na^{+} to enter into the postsynaptic neuron, cause cytotoxic oedema (Gitler *et al*., 2017). **Figure (2.2).**

Metabotropic glutamate receptors (mGluRs) have an extracellular ligand-binding domain, a heptahelical membrane structure, and a c-terminal tail, different from ionotropic glutamate receptors, mGluRs belong to G-protein coupled receptors (GPCRs), Group 1 mGluRs (Gp1 mGluRs) consist of two members, mGluR1 and mGluR5. The locations of Gp1 mGluRs in the central nervous system (CNS) vary from isoform to isoform (Alvarez and others 2000). mGluR1 is mainly expressed in the hippocampus, cerebellum, and substantia nigra, meanwhile mGluR5 is expressed in the hippocampus, amygdala, olfactory bulb, striatum, nucleus accumbens, septum, and dorsal horn (Abe and others 1992; Hubert and others 2001; Jia and others

1999; Martin and other 1992; Romano and others 1995; Ryo and others 1993; Swanson and others 2005; Fig. 1). At synapses, mGluR1 and mGluR5 are mainly located at postsynaptic terminals ,although mGluR5 is also found at presynaptic site and mediates the depletion of phosphatidylinositol-4,5-bisphosphate (PIP2; He and others 2019).

2.2.1 Glutamate transporters at the blood–brain barrier:

The nervous system isolates itself from blood by means of barriers (Alvarez *et al*. 2014).This is important for a number of reasons; one of them is the fact that glutamate Which is orders of magnitude higher than the concentrations that are toxic to neurons (Zlotnik *et al*., 2011).

The blood–brain barrier is between blood and the interstitial fluid of the brain. It is in mammals formed by the endothelial cells after influence from brain cells. Another barrier is in the choroid plexus epithelium which secretes cerebrospinal fluid (CSF) these barriers are important both from a physiological point of view because they are essential for brain homeostasis, and from a pharmacological point of view because they prevent drugs from entering brain tissue(Deboer&Gaillard, 2007; Teichberg *et al.,* 2007).There were, however, huge amounts of glutamate transporters in the astrocytic end feet surrounding the blood vessels when isolating brain microvessels, the preparations are likely to be contaminated by end feet and this may explain some of the data Thus, it seems that no significant transport of glutamate can occur through a normal and intact blood–brain barrier in agreement, injection of radiolabeled glutamate and aspartate does not result in accumulation of radioactivity in the brain (Klin *et al*. 2010).

There is an efflux mechanism for glutamate as blood-mediated scavenging is reported to reduce glutamate in the cerebrospinal fluid (Gottlieb *et al*. 2003).There was some evidence that this may offer some protection (Nagy *et al*., 2010). The mechanism, however, of release from the brain remains to be identified this illustrates that brain water homeostasis and transport mechanisms between the blood and the extracellular fluid in brain are incompletely understood recent work from Nedergaard and co-workers may represent a leap in our understanding they introduce the term

"glymphatics" (Iliff *et al.,* 2012; Nedergaard *et al.,* 2013). After release into the synaptic cleft, excessive glutamate must be cleared quickly to prevent neurotoxicity there are no extracellular enzymes that can neutralize glutamate (Schousboe *et al.,* 2014). However, astrocytes can rapidly clear glutamate from the synaptic clefts via astrocytic processes that completely enclose many glutamatergic synapses these astrocytic processes also express highly efficient excitatory amino acid transporters (Chung WS &Allen NJ, 2015).

That take up 80% of extracellular glutamate in the CNS (Verkhratsky *et al.,* 2018).Once inside the astrocyte, it is estimated that ~85% of glutamate is converted into glutamine and returned to neurons, while the remaining ~15% is metabolized to α-ketoglutarate and further oxidized through the tricyclic acid cycle for energy production this helps to cover the energy costs of glutamate handling as both pyruvate carboxylase and glutamine synthetase catalyze energy-dependent reactions (Conradi *et al.,* 2013).

2.2.2. Alzheimer's disease

Alzheimer's disease (AD) is the most common neurodegenerative disorder that causes dementia and affects middle to old-aged individuals (Hager *et al.,* 2001; Gonzalez *et al.,* 2014; Irwin *et al.,* 2016).AD is characterized by progressive loss of cognitive functions, including memory, language, and reasoning (Di Domenico *et al*., 2015). Studies have investigated the effects of ALA in experimental AD models (Sancheti *et al*., 2013).

Evaluated the chronic dietary supplementation with ALA on hippocampus dependent memory of aged Tg2576 rats, a transgenic model of cerebral amyloidosis associated with AD,ALA treatment to reduce hippocampal was shown -dependent memory deficits, significantly improving learning and memory in the Morris water maze in comparison to rats that did not receive ALA however, no significant differences in b-amyloid levels were found between rats, that received ALA in comparison to the ones that did not receive ALA, indicating that chronic ALA supplementation in the diet can ameliorate hippocampal memory impairments in rats without any effect

on beta amyloid levels or plaque deposition another study assessed the effects ALA in senescence accelerated mouse prone8 (SAMP8) rat, associated to learning and memory impair, and showed that ALA can ents improve memory, in different paradigms (Farr *et al*., 2012).

2.2.3. Antioxidant defenses:

Antioxidants are molecules or compounds that act as free radical scavengers; most antioxidants are electron donors and react with the free radicals to form in innocuous end products such as water these antioxidants bind and inactivate the free radicals, thus antioxidants protect against oxidative stress and prevent damage to cells there are many examples of antioxidants (Dhivat *et al* ., 2001). Such as superoxide dismutase (SOD),glutathione peroxidase reduced glutathione (GSH), sulfhydryl groups , alpha lipoic acid CoQ10 , thioredoxin essential nutrients , vitamin C , vitamin E, selenium, Nacetly cysteine (NAC), in addition to dietary compounds such as bioflavonoids and proanthocynaidans (Beauchamp &Renier , 2002; Lin *et al*. , 2002 ; Osturk *et al*. , 2002 ; Mc-Nulty *et al*., 2006) .

Generally antioxidants are classified to in two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipid (hydrophobic) water soluble antioxidant reacts with oxidants in the cell cytoplasm and blood plasma while hydrophobic antioxidants protect cell membrane from lipid peroxidation (Asies *et al*., 1997).These compounds may be synthesized in the body or obtained from diet different antioxidant are present at wide range of concentration in body fluids and tissues, such as glutathione or ubiquinone present mostly within the cell, while others such as uric acid more evenly distributed throughout the body (Rensma *et al*., 2003).

Ascorbic acid are examples of water soluble antioxidant, while carotenes and tocopherols (vitamin E), ubiquinone and coenzymes Q are lipid soluble antioxidants (Zita *et al.,* 2006).Another classification divided antioxidant into enzymatic and non-enzymatic antioxidant Superoxide dismutase, catalase and glutathione system which include glutathione reductase, glutathione S-transferases are major endogenous antioxidant system in the body while antioxidant proteins, transferrin, ceruloplasmin, and vitamins A, C, E and Beta carotenes are examples of non-enzymatic antioxidants (Chen *et al.,* 2006).

2.3. Alpha Lipoic Acid:

2.3.1 Alpha lipoic acid (ALA)

ALA is a specific antioxidant; it can easily quench radicals, has an amphiphilic character, and does not exhibit any serious side effects (Gora *et al*., 2011). ALA compound that contains sulfur in the form of two thiol groups, ALA is also called thioctic acid and chemically symbol (C8H14O2S2) with an oxidized (disulfide, LA) and with a reduced (di-thiol: dihydro-lipoic acid, DHLA) form of LA as show in **figure (2.3)** (Rochette et al .,2015) .

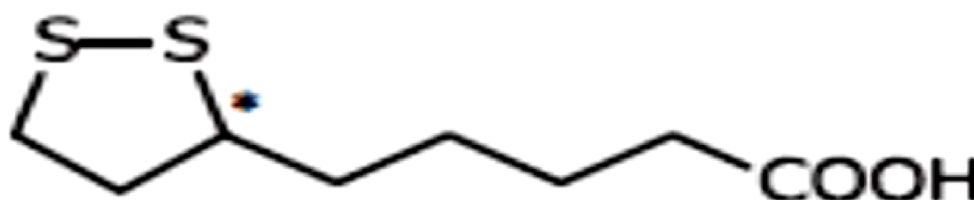

Figure (2.3) Structure Alpha Lipoic Acid (Rochette *et al .,* 2015) .

ALA acts as a cofactor for several mitochondrial enzymes by ability to directly scavenge ROS, its metal chelating activity, and its potential to react with, and regenerate,other antioxidants such as glutathione and vitamins E and C (Singh & Jialal , 2008).

ALA also demonstrates anti-inflammatory properties an additional advantage of ALA is its solubility both in water and in fat, which allows it to travel to all parts of the body because of its special properties, it is able to enter certain parts of the cell that most other antioxidants are not able to reach this compound acts by many mechanisms and can therefore be a very effective antioxidant. Hence, ALA is used in various diseases concerning age-dependent oxidative stress it can be particularly effective in cardiovascular diseases, including ischemic heart disease, hypertension, heart failure, and atherosclerosis, where it may slow aging and prolong lifespan many study have confirmed that ALA can improve vascular function and decrease the atherosclerotic plaque burden (Wollin &Jones, 2003).

ALA inhibits the vascular overproduction of endothelin I, the main vasoconstrictor (Takaoka *et al.*, 2001). Furthermore, ALA significantly increases the synthesis of NO-, the main vasodilator; it may also improve the redox state of the plasma and improve endothelium-dependent NO-mediated vasodilation in addition, ALA ameliorates the loss of ENOS phosphorylation, which contributes to improve endothelial Function ALA act as a defensive agent versus risk factors of cardiovascular disease and may influence the CVD risk *via* the beneficial actions on LDL oxidation, blood lipid profiles, plaque formation and hypertension (Wollin &Jones, 2003).

ALA has the ability to scavenge ROS, metal chelating, and regenerate, glutathione, vitamins E and C (Singh & Jialal, 2008).ALA also has anti-inflammatory properties ALA can mend vascular function and decrease the atherosclerotic plaque burden DHLA, the reduced form of ALA, is capable of exerting an antioxidant effect directly by donating electrons to a pro-oxidant or an oxidized molecule It can regenerate ascorbic acid from dehydroascorbic acid, and it can indirectly regenerate vitamin E back from its oxidized state as well, ALA metabolites have been shown to have antioxidant effects (Kwiecien *et al*., 2013).ALA, the oxidized form of DHLA, can exert an antioxidant effect But this does not mean there is any donation of electrons by ALA to a pro-oxidant or oxidized molecule, since there are none to give however, it has been documented that ALA can inactivate free radicals, which is a significant antioxidant effect (Packer *et al*., 2001).

Also, the ability of ALA to chelate metals can produce an antioxidant effect DHLA can exert a pro-oxidant effect of donating its electrons for the reduction of iron, which can then break down peroxide to the prooxidant hydroxyl radical *via* the Fenton reaction thus, ALA and its reduced form DHLA, can promote antioxidant properties ALA has been effectively chelate toxic metals directly, and it also indirectly strongly supports the chelation of metals by its ability to increase glutathione levels inside the cells (Ghibu *et al*., 2009).

2.3.2 Synthesis of Alph Lipoic Acid:

(ALA) is an amphipathic substance, which is synthesized in the mitochondria of plants and animals from octanoic acid and cysteine as a sulfur donor through the reactions catalyzed by the enzyme lipoic acid synthase the participation of ALA in

oxidative metabolism is essential (Chanwitheesuk *et al*., 2005).ALA chemically exists in two enantiomeric forms R and S although only the R isoform acts as a cofactor in the oxidant metabolism, since it binds through an amide bond to the amino group of the lysine residues this allows it to form a lipoamide, which is a cofactor of the enzymes, pyruvate dehydrogenase and α-ketoglutaratedehydrogenase (Padmalayam *et al*., 2009).Several study have demonstrated the antioxidant, anti-inflammatory, and hypoglycemic properties of ALA (Packer *et al*., 2001 ; Gorąca *et al*., 2011).Furthermore, ALA has been shown to have a positive the OxS linked with aging (Patel *et al*., 2014).

(ALA) play principle role in antioxidant defense in the brain because of their roles as biologic antioxidants Also, it was previously shows that ALA has neuroprotective effects in experimental brain injury caused by trauma and subarachnoid hemorrhage (Toklu *et al*., 2009; Ersahin *et al*., 2010).

(ALA) is well known antioxidant, and has ROS scavenging capacity, the capacity to regenerate endogenous antioxidants such as glutathione, and vitamins E and C, and a metal chelating capacity Besides, ALA is both water and lipid soluble and therefore can cross biological membranes easily, such as blood–brain barrier, and performs its antioxidant action both in the cytosol and in the plasma membrane (Gorąca *et al*., 2011).There were numerous studies that confirmed antioxidative effects ALA in many different organs and tissues (Miao *et al*., 2013; Fernández-Galilea *et al*., 2012). and also neuroprotective effects (Bramanti *et al*., 2010).

2.4. Behavioral Tests:

2.4.1 Forced Swimming Test (FST):

Depression is a life-threatening psychiatric disorder and a major public health concern worldwide with an incidence of 5% and a life time prevalence of 15-20% Moreover, it is estimated that by 2020 depression will be in the top three contributors to the burden of disease (Levinson *et al*., 2006). Depression is associated with disability, decreased quality of life, increased health-related costs and is considered a main risk factor for many diseases, including cardiovascular, metabolic and neuropsychiatric disorders (Cryan *et al*., 2005).Current pharmaco-therapeutic treatments have limited efficacy and are associated with many deleterious side effects (Lam *et al*., 2004; Dording *et al*., 2002).Therefore, a better understanding of the pathophysiology

of this disorder alongside with the development of innovative and improved treatments remains crucial. Hence, animal models are essential for advancing research in this field there are many models used for the study of this disorder (e.g. sucrose preference test, tail suspension test) with the forced swimming test (FST, also known as Porsolt's test after the developer of this model being one of the most commonly used assays (Doron et *al*., 2014).

During the FST an animal is placed in a container filled with water from which it cannot escape the animal will first try to escape but eventually will exhibit immobility (i.e. floating with the absence of any movement except for those necessary for keeping the nose above water) the FST is a very popular model in animal research for a number of reasons first, it involves the exposure of the animals to stress, which was shown to have a role in the tendency for major depression (Doron *et al*., 2014).Moreover depression is often viewed as a lack of ability to handle with stress (Anisman &Zacharko, 1990; Sullivan *et al*., 2000).Second, pharmacological treatment with antidepressants prior to the test has been shown to reduce immobility in the FST (Cryan *et al*., 2005).

2.4.2 Light/Dark Test(LDT)

The light/dark Test (LDT) is based on an approach-avoidance conflict between exploration of novel environments and avoidance of brightly lit, open spaces (Crawley *et al*., 1985).The test was developed in rat by Crawley and colleagues, who observed that anxiolytic drugs increased the number of crossings between compartments (Crawley *et al*., 1980; Crawley *et al*., 1981).Later study showed that time in the light compartment and distance traveled in the light also reflect anxiety-like behavior and expanded the use of the LDT to rats (Bourin &Hascoet, 2003).

The LDT has been widely used to assess anxiety-like behavior in adult rodents, and a few studies have utilized this test in younger animals risk taking behavior peaks during adolescence and contributes to most of the major causes of adolescent injury and mortality (Steinberg *et al*., 2008; Eaton DK & Kann L, 2012). Immature function of corticolimbic and neuromodulatory systems mediating avoidance of aversive stimuli is thought to contribute to these changes in adolescent behavior (Vasa *et al*., 2011).

Behavior in unconditioned anxiety tests such as the LDT is thought to reflect impulsivity or risk taking in addition to anxiety, thus, the LDT may be a useful model to investigate neural systems relevant to adolescent risk taking (Harro *et al.*, 2002).Typically, male rat are used for behavior experiments, as variations of estrous cycle might influence the performance of females in such tests (Simpson *et al.*, 2012).

2.4.3 Elevated Plus Maze Test (EPT)

The elevated plus-maze (EPM) is a common, and per-haps the most popular, behavioral test used in the study of fear and anxiety fear and anxiety, whether produced by a painful stimulus, a predator, or even exposure to bright light produces freezing behaviour in many species in the case of mice and rats, freezing behavior and avoidance of brightly lit areas are used as measures to assess anxiety and fear in behavioral tests such as the open field test the EPM is based on the conflict between the natural tendency of mice and rats to explore a novel environment balanced against the aver-sive and anxiety producing properties of entering a brightly-lit open area. (Hogg *et al.*, 1996; Rodgers &Dalvi, 1997).

Animals are provided the opportunity to explore a novel environment consisting of an elevated maze with two intersecting arms; a "closed" arm with tall walls creating an alley that intersects with an 'open' arm without walls that presents an unprotected walking surface normal exploratory behavior is generally concentrated in the closed arms administration of anxiety-provoking compounds leads to a decrease of time spent in the open areas while anxiolytic compounds tend to reduce the aversion to the open arms and increase the exploratory time spent in the open arms the EPM is commonly used by the pharmaceutical industry as a screening tool for compounds with anxiolytic potential (Hogg *et al.*, 1996).In addition, the elevated plus-maze has been proposed as one of a number of behavioral tests for phenotyping genetically modified rat (Crawley *et al.*, 2003-. Brown *et al.*, 2000).

2.4.4 Morris Water Maze Test (MWT)

Many water mazes have been developed, but the one that is referred to as 'the water maze' was developed by richard morris (Morris *et al*., 1981). The maze was designed as a method to assess spatial or place learning and herein will be referred to as the morris water maze (MWM) morris described the basic procedures in 1984(Morris *et al*., 1984).Subsequently added details and procedures for assessing related forms of learning and memory (Stewart& Morris, 1993).Several characteristics have contributed to the prevalent use of the MWM these include the lack of required pre-training, its high reliability across a wide range of tank configurations and testing procedures, its cross-species utility (rats, mice and humans (Kallai&Makany, 2005).Extensive evidence of its validity as a measure of hippocampally dependent spatial navigation and reference memory (Morris *et al*., 1993).

Its specificity as a measure of place learning, and its relative immunity to motivational differences across a range of experimental treatment effects that are secondary to the central purpose of the task (genetic, pharmacological, nutritional, toxicological and lesion) although the latter is a general characteristic shared by (Cravens *et al*., 1974). The MWM capitalizes on this strength. For example, hippocampal and septohippocampal lesions in rats reliably induce hyperactivity, but such animals show deficits in the MWM (Morris *et al*., 1982). The opposite pole, treatments that induce hypoactivity can be dissociated from learning deficits in the MWM For example, it has been shown that MWM learning impairments are independent of locomotor effects because land-based locomotor reductions did not affect swimming speed Moreover when the experimental animals have deficits during probe trials, this further dissociates learning from performance because measures recorded on probe trials are insensitive to swimming speed (Fitzgerald *et al*., 1989).

2.5. Biochemical parameters:

2.5.1 Beta amyloid:

Beta amyloid peptides of 36–43 amino acids that are the main component of the amyloid plaques found in the brains of people with Alzheimer's disease (Hamley *et al*., 2012). βA is the main component of amyloid plaques, extracellular deposits found in the brains of people with Alzheimer's disease, and can also form the deposits that line cerebral blood vessels in cerebral amyloid angiopathy (Sadigh-Eteghad *et al*., 2014; Röhr *et al*., 2020).

2.5.1.1 Beta amyloid synthesis

Amyloid precursor protein (APP) is a single-pass transmembrane protein which is expressed at high levels in the brain and metabolized in a rapid and highly complex fashion d synthesis (O’brien et al., 2011).

2.5.1.2. Non-amyloidogenic processing:

It is characterized by the non-production of βA peptide APP is cleaved by the enzyme α-secretase within the βA domain, preventing the formation of βA peptide and releasing the soluble ectodomain of APP (sAPP-α) the remaining C-terminal fragment (CTF-α) is subsequently cleaved by γ-secretase, liberating the non-toxic peptide P3 and the APP intracellular domain (AICD) as shown in Figure 2.4 (Grimm *et al*., 2013; Dawkins & Small, 2014).

2.5.1.3. Amyloidogenic processing:

Is characterized by the production of βA peptide APP is cleaved by the enzyme β-secretase within the extracellular domain, at position 671, shedding off the soluble ectodomain of APP (sAPP-β)the remaining C-terminal fragment (CTF-β) is subsequently processed by γ-secretase, releasing the Aβ peptide and the AICD fragment as shown in **Figure (2.4)** (Thinakaran & Koo, 2008; Grimm *et al*., 2013).

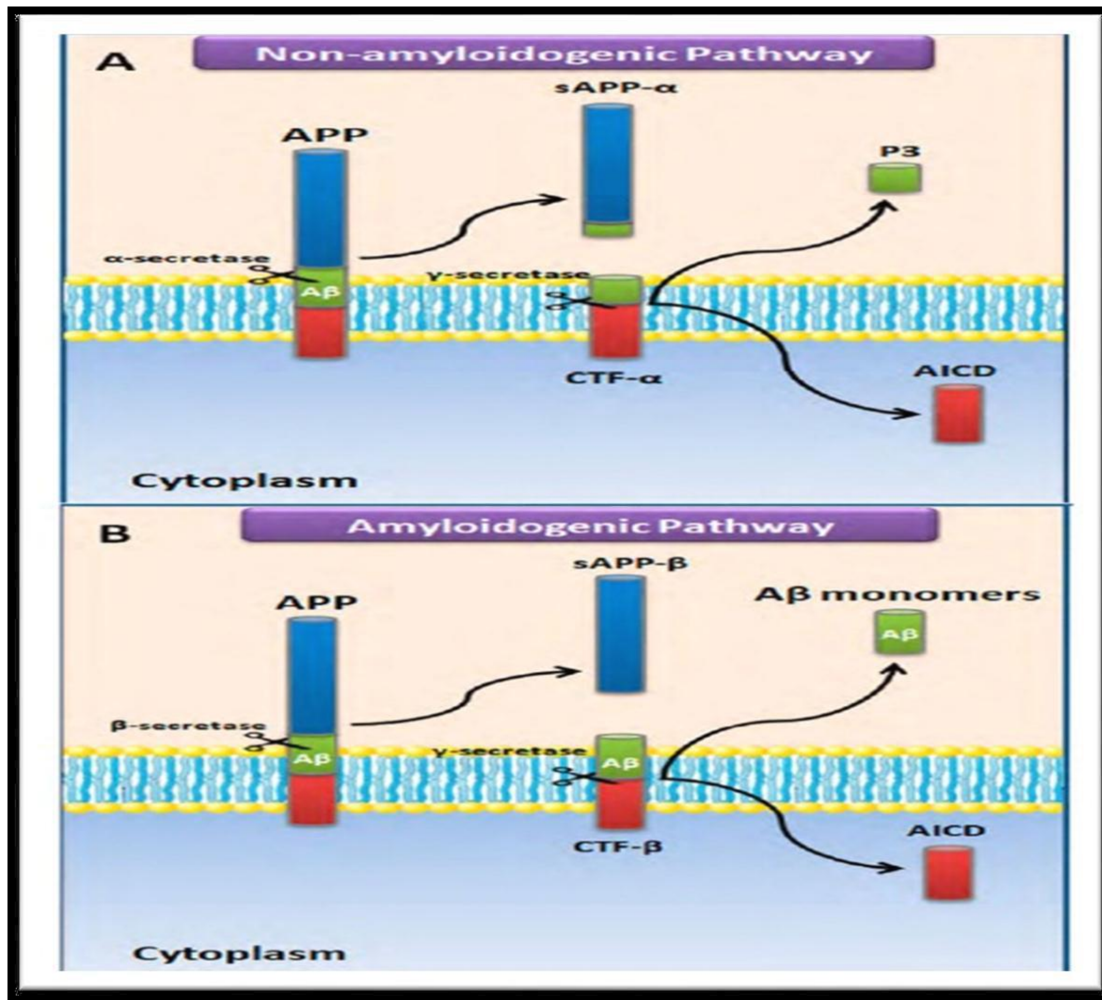

Figure 2.4 Proteolytic processing pathways of APP (Liste *et al*, .2019).(A) Non-amyloidogenic pathway, where α-secretase and γ-secretase act, generating the proteolytic derivatives sAPP-α, p3 and AICD (B) Amyloidogenic pathway, where β-secretase and γ-secretase act, generating the proteolytic derivatives sAPP-β, βA and AICD APP: Amyloid precursor protein; AICD: amyloid precursor protein intracellular domain; Aβ: amyloid-β; sAPP: soluble amyloid precursor protein; CTF: C-terminal fragment.

2.5.1.4. Acetylcholinesterase (AChE):

Acetylcholinesterase is known to be distributed in nervous tissue such as the brainstem, cerebellum, peripheral and autonomic nervous systems skeletal muscle also contains AChE with distribution patterns seemingly related to the type of muscle (fast versus slow twitch) and their specific function (Brimijoin &Molecular, 1983). The presence and function of AChE on red blood cells is less commonly known blood

group antigens reside on the outer lipid bilayer of red blood cells for convenient anti-body recognition. In the same regard, AChE is also present on red blood cell membranes (Bartels & Zelinski, 1993).

2.5.1.5 Acetylcholinesterase Mechanism:

The interaction of acetylcholinesterase with the substrate acetylcholine results in the breakdown, hydrolysis, and inactivation of acetylcholine and subsequent control of the amount of AChE at the synapse AChE is a serine hydrolase that creates a tetrahedral intermediate through acid-base reactions with a catalytic triad (serine, histidine, acid residue) (Soreq& Seidman, 2001). Histidine allows for the transference of a proton between the oxygen molecules in serine and AChE, thereby removing choline to form a new acylated serine,also in when the acylated serine is deacylated, the regeneration of free AChE begins, also in this reaction, aspartate stabilizes the protonated histidine, which releases acetic acid and a new, free enzyme the interaction between amino acid residues (tyrosine, phenylalanine, and tryptophan) that make up a peripheral anionic site influences the conformational binding of AChE to that site. (McHardy &Wang, 2017).

Chapter Three: Methodology

3. Materials & Methods

3.1. Materials

3.1.1. Instruments and Equipment:

All the devices utilized as a part of this study are summarized in **Table 3.1**

Table 3.1. Apparatus and equipment with their manufactures.

NO.	Apparatus & Equipment	Company	Manufactures
1.	natomical set(Scissors, Forceps, Scalpel)	Chemo lab	China
2.	Balance	Denver	Germany
3.	Beakers (100, 250, 500, 1000)	Chemo lab	India
4.	Centrifuge	Hettich	Germany
5.	Colony flask	Chemo lab	India
6.	Cotton	Entrepreneur	India
7.	Digital balance	Denver	Germany
8.	Digital camera	Canon	China
9.	Eppendorf's tubes	Chemo lab	India
10.	Filter paper	Chemo lab	India
11.	Gel tube	Chemo lab	India
12.	Insulin syringe	eldawlia	Egypt
13.	Pipette tips (10 – 1000) µl vol-ume	Chemo lab	India
14.	Sensitive balance	Sartorius	Germany
15.	Surgical gloves	Chemo lab	India
16.	Syringe (1 ml, 5 ml)	Chemo lab	India
17.	Test tubes	Chemo lab	India
18.	Freezer	Hitachi	Japan
19.	Mince machine	Hitachi	Japan
20.	Beaker	Chemo lab	India

3.1.2. Chemicals and Kits all the chemicals and the standard kits used in this study are shown in **Table 3.2.**

Table 3.2: Chemicals and Kits with their suppliers.

No.	Chemicals & Kits	Company	Suppliers
1.	D-galactose	Thomas Baker	India
2.	Acetylcholine Esterase	Elabscience	USA
3.	Beta amyloid	Elabscience	USA
4.	Chloroform	Scharlau	Spain
5.	Glutathione(GSH)	laboratorio ct	Italy
6.	Total Antioxidant Capacity	Elabscience	USA
7.	Alpha lipoic acid	Elabscience	USA
8.	DMSO	LOBA	China
9.	Malondialdehyde (MDA)	Elabscience	USA
10.	SuperoxideDismutase(SOD)	Elabscience	USA
11.	Catalase (CAT)	Elabscience	USA
12.	Perchloric acid	SdfcL	India
13.	Acetic anhydride	SdfcL	India
14.	Glacial acetic acid	SdfcL	India

3.2. Examination methods:

3.2.1. Experimental protocol

Forty (40) white male rats weight (200-220g) were used in the current study taken from the college of Pharmacy University of Kerbala –Iraq and their ages between (12-15) weeks and the animals were placed in good condition in special plastic cages. They are provided with an appropriate conditions and ventilation. The light system was 12 hrs. per day with a relative humidity of 50±5%. They were kept for 2 weeks for adaptation with standard experimental condition. The experiment start at the 30th of October and ended at the 30th of November. Temperature was maintained at (21-25) C° Using a room thermostat the air of the room was changed continuous by using ventilation vacuum and animal fed on the pellet of freshly prepared ration.

3.2.1.1. Experimental Design

Forty (40) white male rat randomly divided into equally four (4) groups (10/group) and treated as follows for (30 days) (***Figure 3.1***).

1-Control: - Rats of this group were injected with normal saline IP, serving as control.

2-D-gal: - Rats of this group were injected IP 200mg/kg B.W. of D-gal (Zhang, Y., et *al*., 2018).(30 days)

3-ALA: - Rats of this group were injected IP 100mg/kg B.W. of ALA (Li *et al*., 2015).(30 days)

4-D-gaI+ ALA: - Rats of this group were injected IP 200mg/kg B.W. of D-gal and injected IP 100mg/kg B.W. of ALA (30 days)

3.2.1.2. Collecting of the blood samples

Fasting Blood samples were drawn after 30 days of the experiment, the animals anesthetized by chloroform Inhalation in order to control and calm the animal before the blood draw. 5 ml blood was drawn from the heart by heart puncture technique and sterile medical syringes of 5 ml were used, then the blood was placed In special gel tube not containing an anticoagulant, the serum was separated by a centrifuge at a speed of 3000 r / min for 5 minutes, the separated serum put in a Eppendorf's tubes and kept in freeze at -20 ° C until the completion of the measurements.

3.2.1.3. Collecting of the brain samples

Brain samples were weighed, and homogenized for 150 seconds (to ensure the tissue was well dispersed) in 0.1 N perchloric acid (200 µl), using Squishers. The homogenates were then centrifuged in a cold room (4ºC) for 30 minutes, and the su-pernatants drawn off carefully. At this stage the supernatants could be frozen (-80ºC) or taken directly for analysis .Take 500ml from anhydral glacial acetic acid and Add 25ml from acetic anhydride and Add about 8.5 ml from perchloric acid and put in cool (24h). Complete the mixture to1000ml from Anhydral glacial acetic acid.
Measurement of the glutamate and beat amyloid.

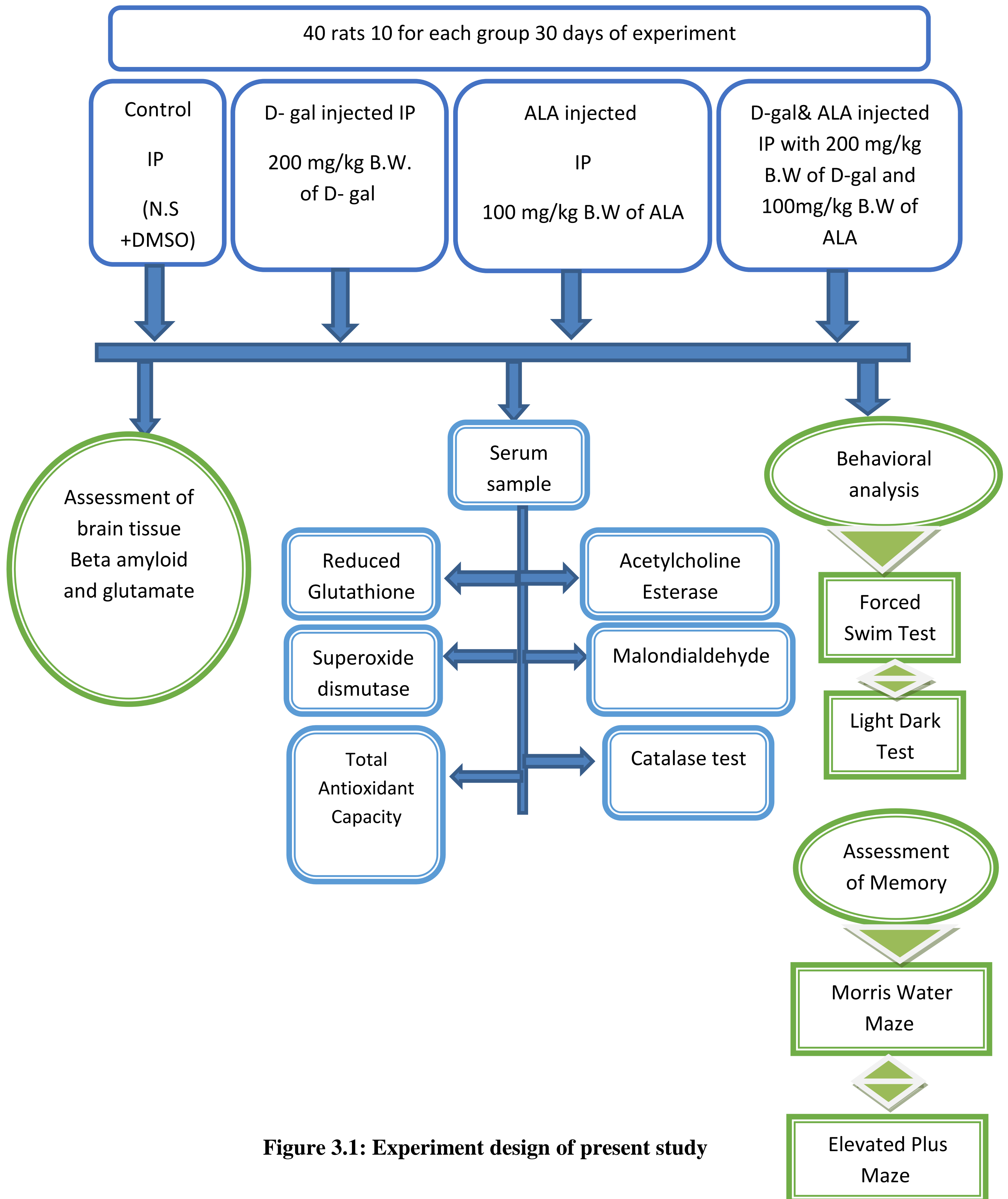

Figure 3.1: Experiment design of present study

3.3 measurement of neruomarker concentration:

The neruometers concentration were measured by using a special Enzyme-Linked Immunosorbent Assay (ELISA) kits, and there were measured by the steps included in the kits.

3.3.1 Measurement of tissue Beta amyloid:

Brain Tissue rat was measured using ELISA kit **(Table 3.2).** This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Rat, as illustrated in **appendices I.**

3.3.2. Measurement of serum Acetylcholine Esterase (AChE) Activity

Serum rat Acetylcholine Esterase (AChE) was measured using ELISA kit **(Table 3.2).**This ELISA kits uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kits has been pre-coated with an antibody specific to Rat AChE, as illustrated in **appendices II.**

3.3.3. Determination of tissue Glutamate of brain study.

The amino acids were extracted according to the method presented by the scientist (Rasmus&Dahl-Lassen,2018).Where a weight of (3 g) was taken from the sample and placed in a volumetric vial with a capacity of (25 ml) and (25 ml) of hydrochloric acid (6M) was added to it at a temperature (150 m) for 3 hours. Then, the sample was dried by the rotary evaporator and (5 ml) of sodium citrate pH 2.2 was added to it the sample was filtered using a plastic filter (0.45um) and taken to the apparatus to perform the injection process as illustrated in **appendices XII.**

3.4. Measurement of biochemical parameters:

3.4.1. Determination of Serum Malondialdehyde (MDA) concentration (µ mol /L):

Malondialdehyde was estimated by Thiobarbituric acid (TBA) assay method of (**Buege & Aust, 1978**) on spectrophotometer, as illustrated in **appendices III.**

3.4.2. Determination of serum Reduced Glutathione concentration:

Reduced glutathione was measured following the method of (Sedlak & Lindsay, 1968), as illustrated in **appendices IV.**

3.4.3. Determination of serum catalase concentration:

Serum catalase concentration was measured following the method of (Hadwan & Abed, 2016), as illustrated in **appendices V.**

3.4.4. Determination of serum concentration of superoxide dismutase (SOD) activity determination:

Serum superoxide dismutase was measured following the method of (Marklund& Marklund, 1974), as illustrated in **appendices VI.**

3.4.5. Determination of serum Total Antioxidant Capacity (TAC)

Serum Total Antioxidant Capacity concentration was measured colorimetrically using Total Antioxidant Capacity Assay Kit (Table 3.2), depending on (Re *et al*., 1999) as illustrated in **appendices VII.**

3.5. Measurement of Behavioral analysis:

3.5.1. Determination of Morris water maze Test (MWM).

Behavior testing was conducted in Morris water maze depending on the source. (Costall *et al.,* 1989). As illustrated in **appendices VIII.**

3.5.2. Determination of Forced Swimming Test (FST).

Behavior testing was conducted Forced Swimming Test in depending on the source (Cryan *et al.,* 2005). As illustrated in **appendices IX.**

3.5.3. Determination of Light/Dark Test (LDT).

Behavior testing was conducted Light/Dark Test in depending on the source (Crawley *et al.*, 1985). As illustrated in **appendices X.**

3.5.4. Determination of Elevated Plus-Maze Test (EPM).

Behavior testing was conducted of Elevated Plus-Maze Test in depending on the source (Hogg *et al.,* 1996). As illustrated in **appendices XI.**

3.6 Statistical analysis:

Data are reported as means ± standard error of the mean (SEM) and data were normally distributed, as tested using the Agostino and Pearson normality test, for comparison between more than two conditions (e.g. drug concentrations), statistical significance was calculated using one-way analysis of variance (ANOVA) followed by Turkey's post hoc tests. The data were analyzed using Graph Pad Prism Version 9.0 for Windows and the criterion for statistical significance is ($P < 0.05$) (Wing *et al.,*2017).

Chapter Four: Results and Analysis

4. Results and Analysis

4.1. Role of ALA on some neruometers on brain damage induced by D-gal in male rats:

4.1.1. Role of ALA on the beta amyloid (pg/ml) on brain tissue damage induced by D-gal in male rats.

The level beta amyloid on brain tissue damage were measured following 30 days experiments the results show that there was a significant increase (P≤0.0001) in d-gal treated group as compared to control group (12.64±3.33).Post-hoc analysis using tukeys multiple comparisons test indicated that treatment with ALA significantly (P≤0.0001) reduced the level of beta amyloid (19.34±1.95) following d-gal induced brain damage as compared to d-gal treated group (28.48±4.47).The mean values at the end of the experiment were (control, 12.64±3.33, D-gal, 28.48±4.47, ALA, 7.06±1.37, ALA+D-gal, 19.34±1.95) for groups control, D-gal, ALA and ALA+D-gal. **Figure (4.1)**

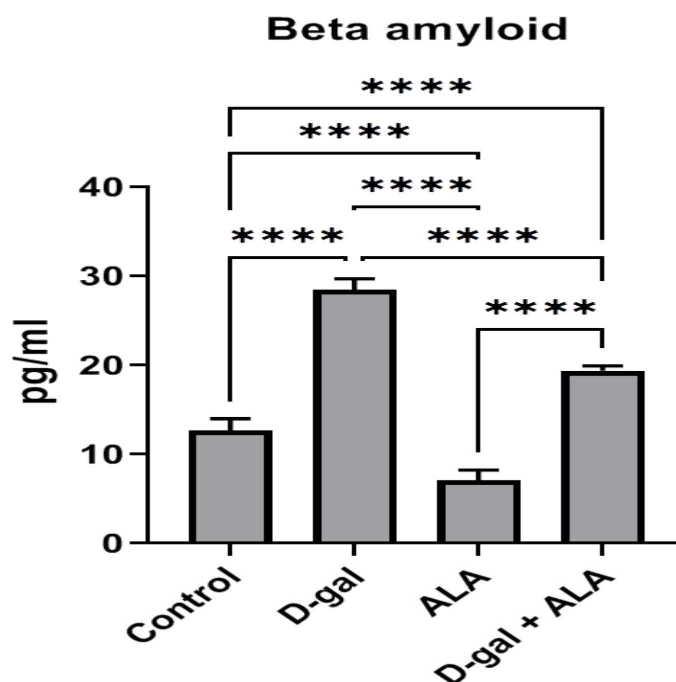

Figure 4.1. Role of ALA on the Beta amyloid (BA) concentration in the brain tissue following D-gal in male rats. There was a significant difference in the of tissue beta-amyloid (BA) in treated groups compare to D-gal group. (****P < 0.0001): values significantly difference from ALA compare to D-gal group. (****P < 0.0001): values significantly difference from D-gal + ALA compare to D-gal group. (****P < 0.0001): Data are expressed as mean ± SEM, n= 5

4.1.2. Role of ALA on the Glutamate in the brain tissue (mol/g) on brain damage induced by D-gal in male rats.

The level glutamate on brain tissue damage were measured following 30 days experiments the results show that there was a significant decrease (P≤0.0001) in d-gal treated group as compared to control group (301.8±0.62).Post-hoc analysis using tukeys multiple comparisons test indicated that treatment with ALA significantly (P≤0.0001)increase the level of glutamate (322.2 ±0.78) following d-gal induced brain damage as compared to d-gal treated group (220.4±0.42).The main value of Glutamate was (control, 301.8±0.62, D-gal, 220.4±0.42,ALA 370.6 ± 1.20,and ALA+D-gal,322.2 ±0.78) for groups control, D-gal, ALA and ALA+D-gal. **Figure (4.2)**

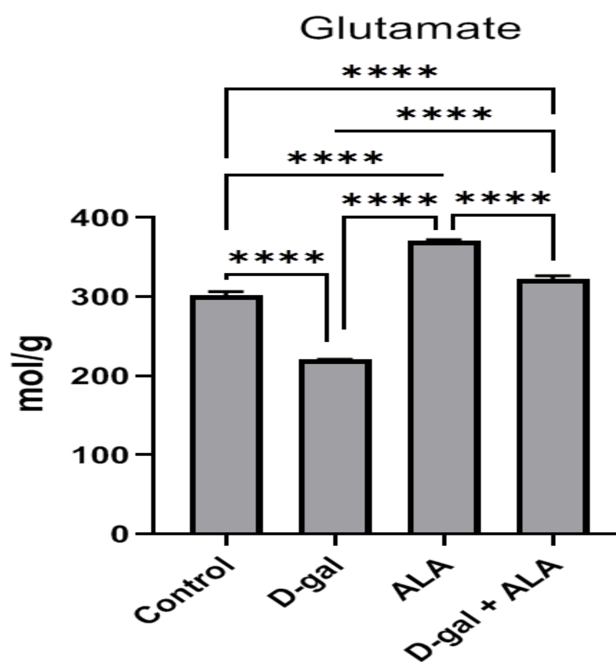

Figure 4.2. Role of ALA on the Glutamate (GIu) concentration in the brain tissue following D-gal in male rats. There was a significant difference in the of tissue glutamate in treated groups compare to D-gal group. (****P < 0.0001): values significantly difference from ALA compare to D-gal group. (****P < 0.0001): values significantly difference from D-gal + ALA compare to D-gal group. (****P < 0.0001): Data are expressed as mean ± SEM, n= 5

4.1.3. Role of ALA on the serum Acetylcholinesterase (AChE) (U/ml) on brain damage induced by D-gal in male rats.

The level serum Acetylcholinesterase (AChE) were measured following 30 days experiments the results show that there was a significant increase (P≤0.05) in d-gal treated group as compared to control group (6.26±0.77).Post-hoc analysis using tukeys multiple comparisons test indicated that treatment with ALA significantly (P≤0.05) reduced the level of Acetylcholinesterase (AChE)(11.03±3.55) following d-gal induced brain damage as compared to d-gal treated group (23.81±2.19).The main value of Acetylcholinesterase was (control, 6.26±0.77, D-gal, 23.81±2.19, ALA, 4.64±0.75, ALA+Dgal, 11.03±3.55) for groups control, D-gal, ALA and ALA+Dgal. **Figure (4.3).**

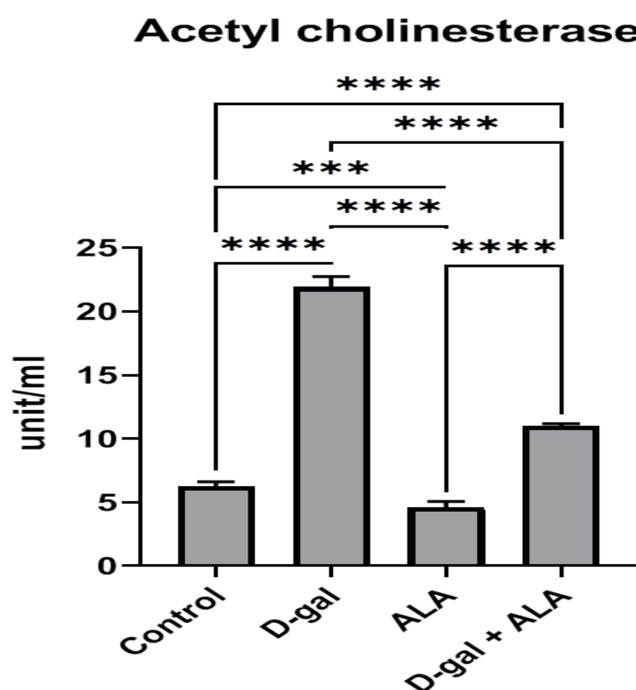

Figure 4.3. Role of ALA on the serum Acetylcholinesterase (AChE) concentration in the brain following D-gal in male rats. There was a significant difference in the level of serum AChE in treated groups compare to D-gal group. (****P < 0.0001): values significantly difference from ALA compare to D-gal group. (****P < 0.0001): values significantly difference from D-gal + ALA compare to D-gal group. (****P < 0.0001): Data are expressed as mean ± SEM, n= 5

4.2. Role of ALA on some oxidant-antioxidant on brain damage induced by D-gal in male rats:

4.2.1. Role of ALA on some serum total antioxidant capacity (TAO-C) (U/mL) concentration on brain damage induced by D-gal in male rats:

The level serum total antioxidant capacity (TAO-C) were measured following 30 days experiments the results show that there was a significant decrease ($P \leq 0.05$) in d-gal treated group as compared to control group (7.58 ± 0.62).Post-hoc analysis using tukeys multiple comparisons test indicated that treatment with ALA significantly ($P \leq 0.02937$) increase the level of Total antioxidant capacity (7.08 ±0.78) following d-gal induced brain damage as compared to d-gal treated group(4.57 ±0.42).The main value of total antioxidant capacity was (control, 7.58 ± 0.62, D-gal, 4.57 ±0.42, ALA, 8.47 ± 1.20, ALA+ D-gal, 7.08 ±0.78) for groups control, D-gal, ALA and ALA+ D-gal. **Figure (4.4)**

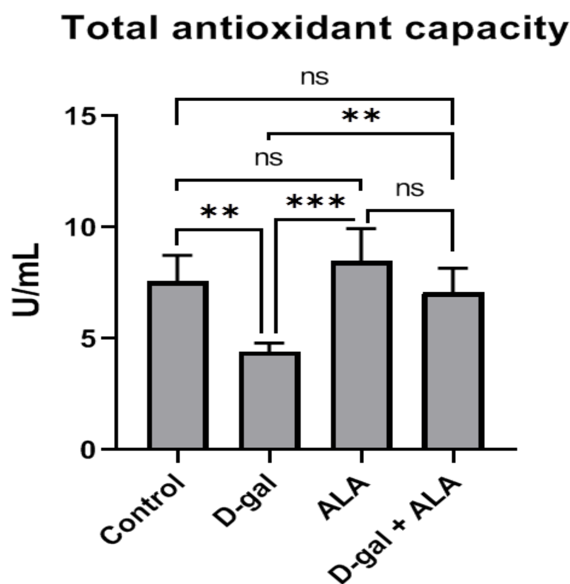

Figure 4.4. Role of ALA on the serum total antioxidant capacity(TAC) concentration in the brain following D-gal in male rats. There was a significant difference in the level of TAC in treated groups compare to D-gal group. (**P < 0.8851): values significantly different from ALA compare to D-gal group. (***P < 0.0001): values significantly difference from D-gal + ALA compare to D-gal group. (**P < 0.08851)

:(ns) refers to values significantly non- difference. Data are expressed as mean ± SEM, n= 5

4.2.2. Role of ALA on serum Glutathione (GSH) (ug/l) concentration on brain damage induced by D-gal in male rats.

The level serum reduced glutathione were measured following 30 days experiments the results show that there was a significant decrease (P≤0.0001) in d-gal treated group as compared to control group (34.72 ± 6.98).Post-hoc analysis using tukeys multiple comparisons test indicated that treatment with ALA significantly (P≤0.0001)increase the level of glutathione (34.67 ±1.24) following d-gal induced brain damage as compared to d-gal treated group (27.50 ± 0.75).The main value of reduced Glutathione was (control, 34.72 ± 6.98, D-gal, 27.50 ± 0.75, ALA, 38.51 ± 3.09, ALA+D-gal, 34.67 ±1.24) for groups control, D-gal, ALA and ALA+D-gal. **Figure (4.5)**

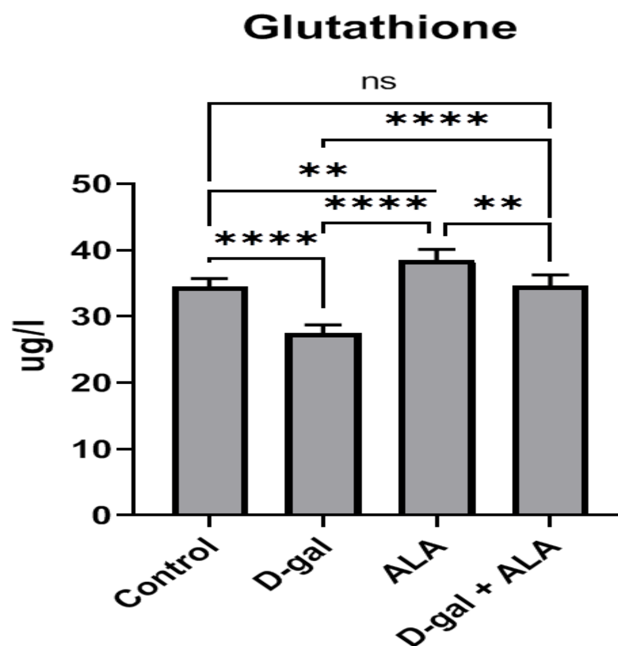

Figure 4.5. Role of ALA on the serum Glutathione (GSH) concentration in the brain following D-gal in male rats. There was a significant difference in the level of serum reduced Glutathione (GSH) in treated groups compare to D-gal group. (****P < 0.0001): values significantly difference from ALA compare to D-gal group. (****P < 0.0001): values significantly different from D-gal + ALA compare to D-gal group.

(****P < 0.0001): (ns) refers to values significantly non- difference.Data are expressed as mean ± SEM, n= 5

4.2.3. Role of ALA on serum Malnodialdehyde (mol/L) concentration on brain damage induced by D-gal in male rats.

The level serum malnodialdehyde were measured following 30 days experiments the results show that there was a significant increase (P≤0.0001) in d-gal treated group as compared to control group (29.21 ± 0.67).Post-hoc analysis using tukeys multiple comparisons test indicated that treatment with ALA significantly (P≤0.0001) reduced the level of malnodialdehyde (32.08 ±1.27) following d-gal induced brain damage as compared to d-gal treated group (38.08 ±2.39).The mean values at the end of the experiment was (control,29.21 ± 0.67, D-gal, 38.08 ±2.39, ALA, 30.70 ± 1.47, ALA+D-gal, 32.08 ±1.27) for groups control, D-gal, ALA and ALA+D-gal. **Figure (4.6)**

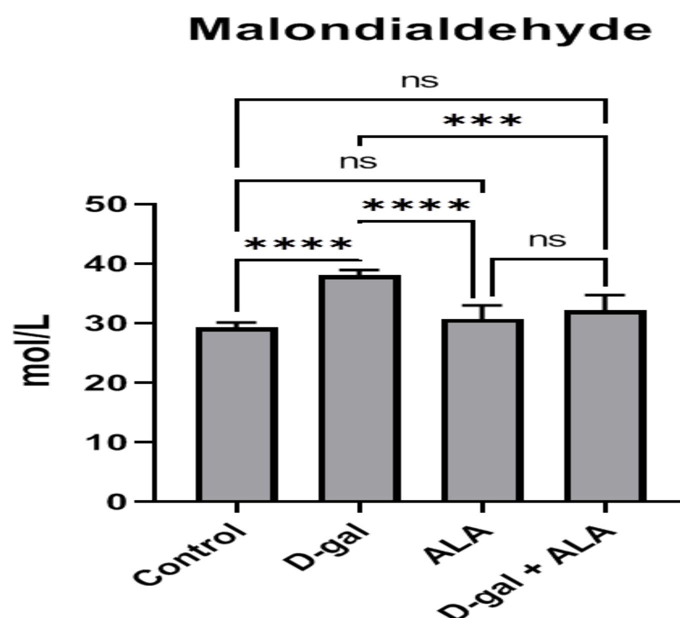

Figure 4.6. Role of ALA on the serum molanodialdehyde (MDA) concentration in the brain following D-gal in male rats. There was a significant difference in the level of serum Melanoaldehyde (MDA) in treated groups compare to D-gal group. (****P < 0.0001): values significantly difference from ALA compare to D-gal group. (****P < 0.0001): values significantly difference from D-gal + ALA compare to D-gal group. (***P < 0.0001):(ns) refers to values significantly non- difference. Data are expressed as mean ± SEM, n= 5

4.2.4. Role of ALA on serum catalase (ku/L) concentration on brain damage induced by D-gal in male rats.

The level serum catalase (CAT) were measured following 30 days experiments the results show that there was a significant decrease (P≤0.05) in d-gal treated group as compared to control group (53.23 ± 6.40).Post-hoc analysis using tukeys multiple comparisons test indicated that treatment with ALA significantly (P≤0.05) increase the level of catalase (47.87±1.93) following d-gal induced brain damage as compared to d-gal treated group (33.42 ±5.05).The mean values at the end of the experiment was (control, 53.23 ± 6.40, D-gal, 33.42 ±5.05, ALA, 55.82 ±4.84, G4 ALA+D-gal, 47.87 ± 1.93) for groups control, D-gal, ALA and ALA+D-gal. **Figure (4.7)**

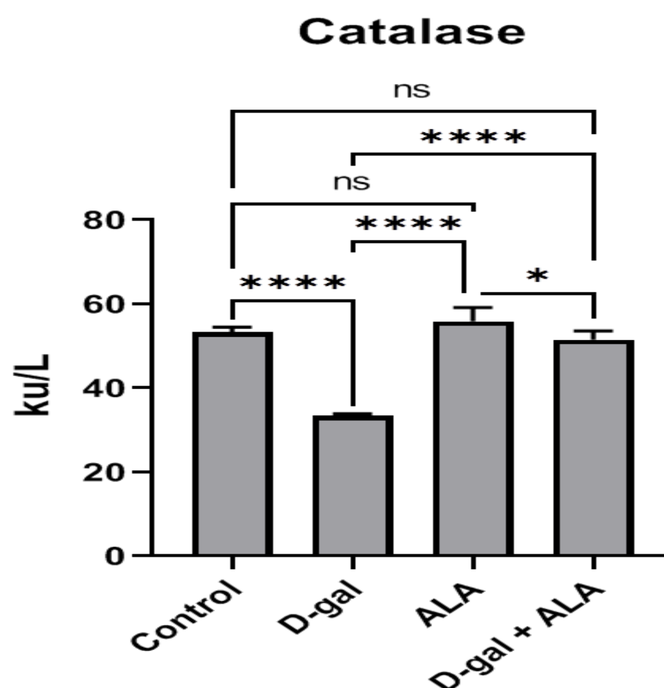

Figure 4.7. Role of ALA on the serum catalase (CAT) concentration in the brain following D-gal in male rats. There was a significant difference in the level of serum catalase (CAT) in treated groups compare to D-gal group. (****P < 0.0001): values significantly difference from ALA compare to D-gal group. (****P < 0.0001): values significantly difference from D-gal + ALA compare to D-gal group. (****P < 0.0001): (ns) refers to values significantly non- difference. Data are expressed as mean ± SEM, n= 5

4.2.5. Role of ALA on serum Superoxide dismutase (U/ml) Concentration on brain damage induced by D-gal in male rats.

The level serum superoxide dismutase (SOD) were measured following 30 days experiments the results show that there was a significant decrease ($P \leq 0.0001$) in d-gal treated group as compared to control group (89.96 ± 3.66).Post-hoc analysis using tukeys multiple comparisons test indicated that treatment with ALA significantly ($P \leq 0.0001$)increase the level of superoxide dismutase (89.22 ± 2.64) following d-gal induced brain damage as compared to d-gal treated group (80.83 ± 3.95). The mean values at the end of the experiment was (control, 89.96 ± 3.66, D-gal, 80.83 ± 3.95, ALA, 94.33±2.40, ALA+D-gal, 89.22 ± 2.64) for groups control, D-gal, AlA and ALA+D-gal. **Figure (4.8)**

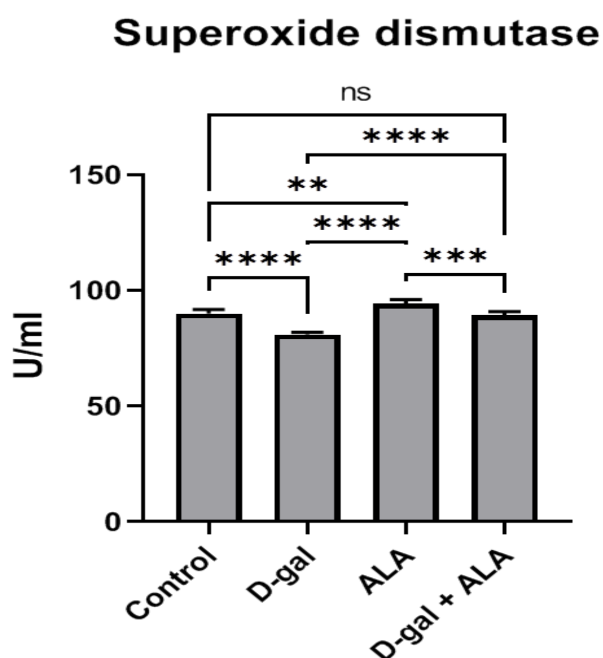

Figure 4.8. Role of ALA on the serum Superoxide dismutase (SOD) concentration in the brain following D-gal in male rats. There was a significant difference in the level of serum Superoxide dismutase (SOD) in treated groups compare to D-gal group. (****$P < 0.0001$): values significantly difference from ALA compare to D-gal group. (****$P < 0.0001$): values significantly difference from D-gal + ALA compare to D-gal group. (****$P < 0.0001$): (ns) refers to values significantly non- difference. Data are expressed as mean ± SEM, n= 5

4.3. Role of ALA on some behavioral analysis on brain damage induced by D-gal in male rats:

4.3.1. Role of ALA on Forced swimming Test (FST) on brain damage induced by D-gal in male rats.

The time forced swim test were measured following 30 days experiments the results show that there was a significant decrease (P≤0.0001) in d-gal treated group as compared to control group (13.2±0.62).Post-hoc analysis using tukeys multiple comparisons test indicated that treatment with ALA no significantly (P≤0.0001) increase the time of forced swim test (10.8±0.78) following d-gal induced brain damage as compared to d-gal treated group (8.6±0.42).The main value of forced swim test was(control,13.2±0.62, D-gal, 8.6±0.42,ALA,13.8±1.20, and ALA+D-gal, 10.8±0.78) for groups control, D-gal, ALA and ALA+D-gal. **Figure (4.9) Image(4.1)**

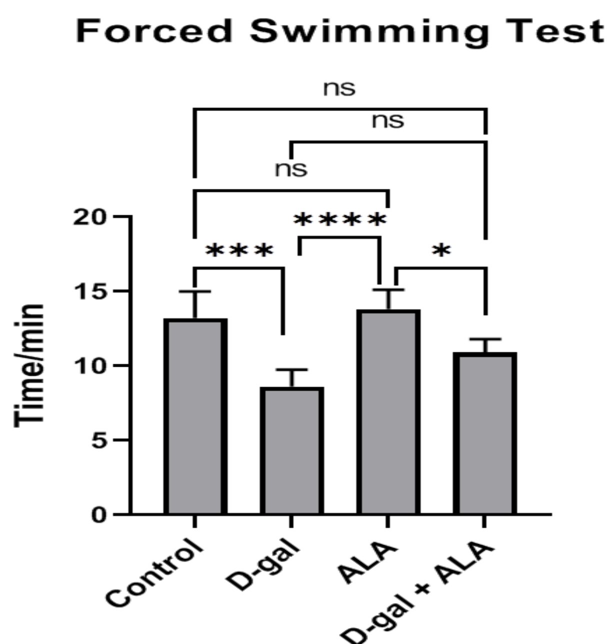

Figure 4.9. Role of ALA on Forced swimming test (FST) in the brain following D-gal in male rats. There was a significant difference in the time of Forced swimming test (FST) in treated groups compare to D-gal group. (***P < 0.0001): values significantly difference from ALA compare to D-gal group. (****P < 0.0001): values significantly difference from D-gal + ALA compare to D-gal group. (ns) refers to values significantly non- difference. Data are expressed as mean ± SEM, n= 5

4.3.2. Role of ALA on Morris Water Maze Test (MWT) on brain damage induced by D-gal in male rats.

The time of morris water maze test (MWM) were measured following 30 days experiments the results show that there was a significant increase (P≤0.0001) in d-gal treated group as compared to control group (34S±1.45).Post-hoc analysis using tukeys multiple comparisons test indicated that treatment with ALA no significantly (P≤0.0001) reduced the time of morris water maze test (72S±8.06) following d-gal induced brain damage as compared to d-gal treated group (79S±10.80).The mean values at the end of the experiment were (control, 34±1.45, D-gal, 79±10.80, ALA, 28±3.79, and ALA+D-gal, 72±8.06) for groups control, D-gal, ALA and ALA+D-gal. **Figure (4.10)Image (4.2)**

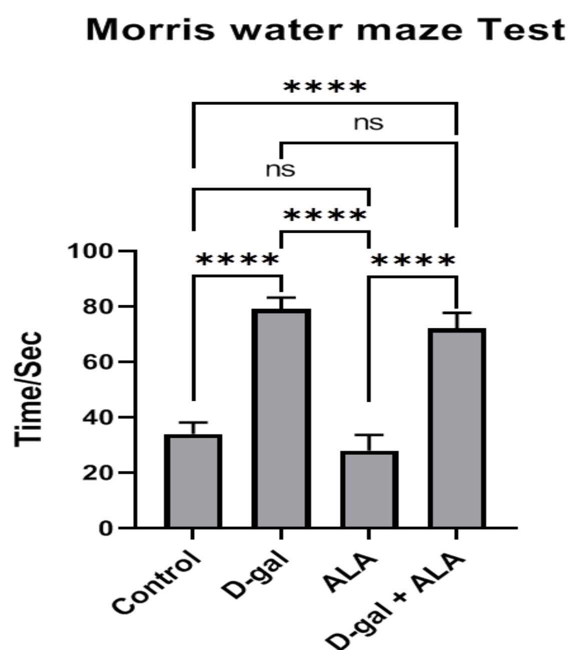

Figure 4.10. Role of ALA on morris water maze test (MWM) in the brain damage following D-gal in male rats. There was a significant difference in the time of Morris water maze test (MWM) in treated groups compare to D-gal group. (****P < 0.0001): values significantly difference from ALA compare to D-gal group. (****P < 0.0001): values was no significantly difference from D-gal + ALA compare to D-gal group. (ns) refers to values significantly non- difference. Data are expressed as mean ± SEM, n= 5

4.3.3. Role of ALA on Dark Room test (DRT) on brain damage induced by D-gal in male rats.

The time Dark Room test were measured following 30 days experiments the results show that there was a significant decrease (P≤0.0001) in d-gal treated group as compared to control group(3.8±0.09).Post-hoc analysis using tukeys multiple comparisons test indicated that treatment with ALA no significantly (P≤0.0001) increase the time of dark room test (1.7±0.57) following d-gal induced brain damage as compared to d-gal treated group (1±0.58). The main value of Dark Room test was (control, 3.8±0.09, D-gal, 1±0.58, ALA, 3.8±0.06, ALA+D-gal, 1.7±0.57) for groups control, D-gal, ALA and ALA+D-gal. **Figure (4.11)**

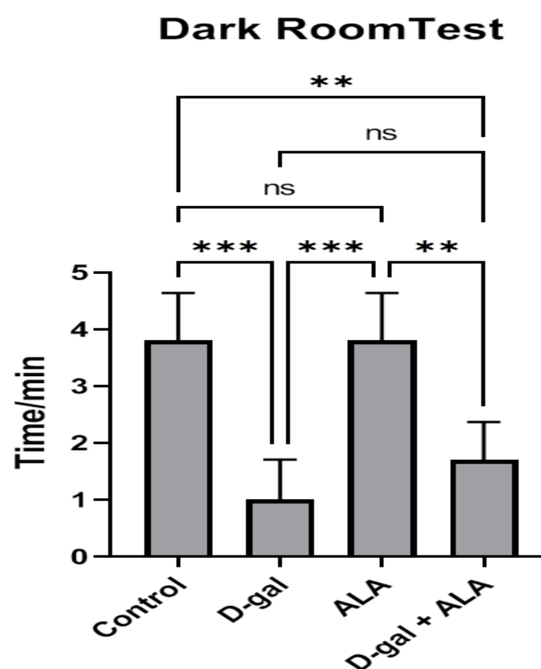

Figure 4.11. Role of ALA on Dark Room test (DRT) in the brain following D-gal in male rats. There was a significant difference in the time of Dark Room test (DRT) in treated groups compare to D-gal group. (***P < 0.0001): values significantly difference from ALA compare to D-gal group. (***P < 0.0001): values was no significantly difference from D-gal + ALA compare to D-gal group. (ns) refers to values significantly non- difference. Data are expressed as mean ± SEM, n= 5

4.3.4. Role of ALA on Light Room test (LRT) on brain damage induced by D-gal in male rats.

The time Light Room test were measured following 30 days experiments the results show that there was a significant increase ($P \leq 0.0001$) in d-gal treated group as compared to control group(1.2±0.09).Post-hoc analysis using tukeys multiple comparisons test indicated that treatment with ALA no significantly ($P \leq 0.0001$) reduced the time of light room test(3.3±0.57) following d-gal induced brain damage as compared to d-gal treated group (4±0.58).The main value of light room test was (control, 1.2±0.09, D-gal, 4±0.58, ALA, 1.2±0.06, ALA+D-gal, 3.3±0.57) for groups control, D-gal, ALA and ALA+D-gal. **Figure (4.12)**

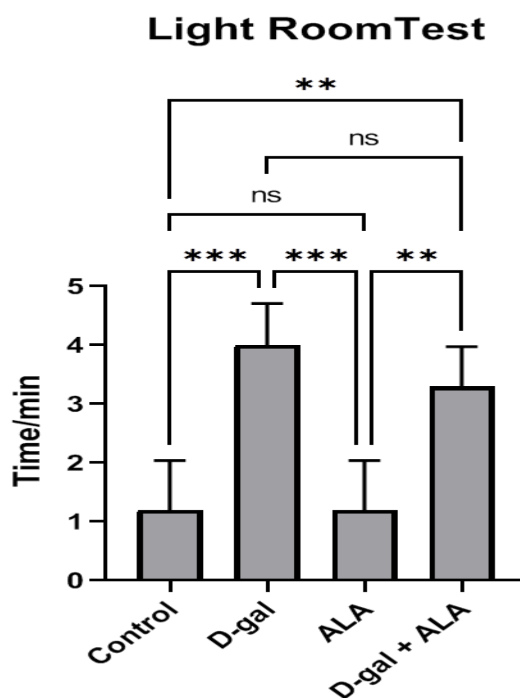

Figure 4.12. Role of ALA on Light Room test (LRT) in the brain following D.gal in male rats. There was a significant difference in the time of Light Room test (LRT) in treated groups compare to D-gal group. (***$P < 0.0001$): values significantly difference from ALA compare to D-gal group. (***$P < 0.0001$): values was no significantly difference from D-gal + ALA compare to D-gal group. (ns) refers to values significantly non- difference. Data are expressed as mean ± SEM, n= 5

4.3.5. Role of ALA on Light& Dark Room test (L&DRT) on brain damage induced by D-gal in male rats.

The time point of light& dark room test in both light& dark room were normalized to the control group. It was nessessary to normalise the data to control group relevaut for each condition rather tham makea direct comparison as the experiment were interestail to determine if the time point affect by each experiment coudition could increase or decrease,using two way analysis of variance (ANOVA) for statistical analysis.The main effect for both condition was statistically significant (P≤0.0001) d-gal and ALA treated groups both condition light& dark rooms.The interaction effect between the conditions and treated groups was statistically significant(P≤0.0001).Post-hoc analysis using tukeys multiple comparisons test indicated that there was significant differeance (P≤0.09636) D-gal, ALA treated group between light& dark rooms.

The main value of light room test was (control, 1.2±0.09, D-gal, 4±0.58, ALA, 1.2±0.06, ALA+D-gal, 3.3±0.57) for groups control, D-gal, ALA and ALA+D-gal. The main value of Dark Room test was(control, 3.8±0.09, D-gal, 1±0.58, ALA, 3.8±0.06, ALA+D-gal, 1.7±0.57) for groups control, D-gal, ALA and ALA+D-gal. **Figure (4.13) Image(4.3)**

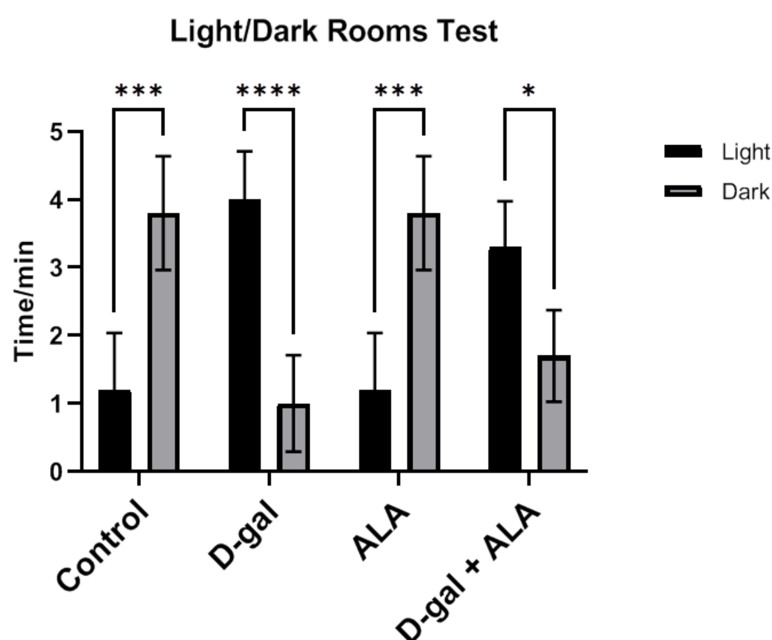

Figure 4.13. Role of ALA on Light& Dark Room test (L&DRT) on brain damage induced by D-gal in male rats. There was a significant difference in the time of

Light& Dark Room test (L&DRT) in treated groups compare to D-gal group. (****P < 0.0001): values significantly difference from ALA compare to D-gal group. (***P < 0.0001): values significantly difference from D-gal + ALA compare to D-gal group. (****P < 0.0001): Data are expressed as mean ± SEM, n= 5

4.3.6. Role of ALA on Elevated plus-maze test \ open arm on brain damage induced by D-gal in male rats.

The time elevated plus-maze test \ open arm were measured following 30 days experiments the results show that there was a significant increase (P≤0.0001) in d-gal treated group as compared to control group (l,2.6±0.80).Post-hoc analysis using tukeys multiple comparisons test indicated that treatment with ALA significantly (P≤0.0001) reduced the time of elevated plus-maze test \ open arm (7.2±1.01) following d-gal induced brain damage as compared to d-gal treated group (8±1.84). The main value of Elevated plus-maze test \ open arm was (control,2.6±0.80, D-gal, 8±1.84,ALA,1.6±0.83, ALA+D-gal,7.2±1.01) for groups control, D-gal, ALA and ALA+D-gal. **Figure (4.14)**

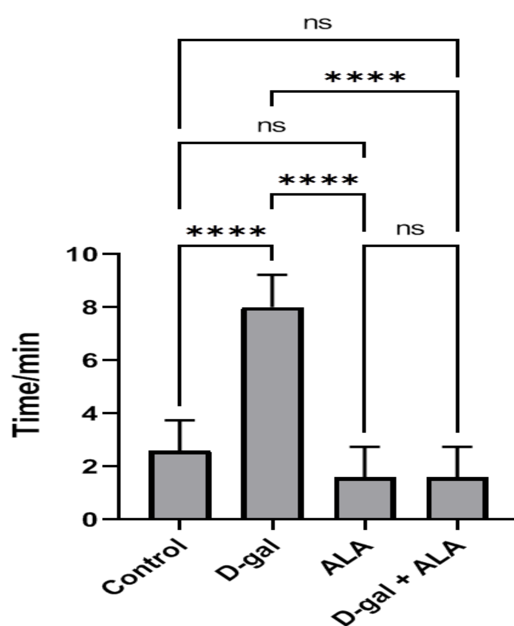

Figure 4.14. Role of ALA on Elevated plus-maze test \ open arm on brain damage induced by D-gal in male rats. There was a significant difference in the time of Elevated plus-maze test \ open arm in treated groups compare to D-gal group. (****P < 0.0001): values significantly difference from ALA compare to D-gal group. (****P < 0.0001): values significantly difference from D-gal + ALA compare to D-gal group. (****P < 0.0001): Data are expressed as mean ± SEM, n= 5

4.3.7. Role of ALA on some Elevated plus-maze test \ closed arm on brain damage induced by D-gal in male rats.

The time elevated plus-maze test \ closed arm were measured following 30 days experiments the results show that there was a significant decrease (P≤0.0001) in d-gal treated group as compared to control group (7.4±0.62).Post-hoc analysis using tukeys multiple comparisons test indicated that treatment with ALA no significantly (P≤0.0001) increase the time of elevated plus-maze test \ closed arm (2.8±0.78) following d-gal induced brain damage as compared to d-gal treated group (2±0.42). The main value of Elevated plus-maze test \ closed arm was (control, 7.4±0.62, D-gal, 2±0.42, ALA, 8.4±1.20, ALA+D-gal, 2.8±0.78) for groups control, D-gal, ALA and ALA+D-gal. **Figure (4.15)**

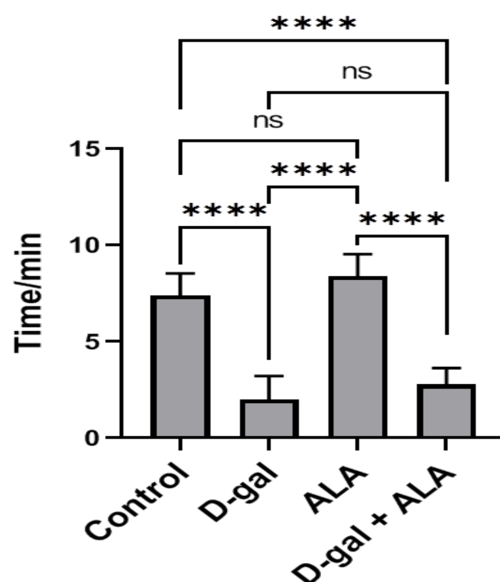

Figure 4.15. Role of ALA on Elevated plus-maze test \ closed arm on brain damage induced by D-gal in male rats. There was a significant difference in the time of Elevated plus-maze test \ closed arm in treated groups compare to D-gal group. (****P < 0.0001): values significantly difference from ALA compare to D-gal group. (****P < 0.0001): values was no significantly difference from D-gal + ALA compare to D-gal group. (ns) refers to values significantly non- difference. Data are expressed as mean ± SEM, n= 5

4.3.8. Role of ALA on Elevated plus-maze test \ open& closed arm on brain damage induced by D-gal in male rats.

The time point of elevated plus-maze test in both open and closed arm were normalized to the control group. It was nessessary to normalise the data to control group relevaut for each condition rather tham makea direct comparison as the experiment were interestail to determine if the time point affect by each experiment coudition could increase or decrease, using two way analysis of variance (ANOVA) for statistical analysis.The main effect for both condition was statistically significant (P≤0.05) d-gal and ALA treated groups both condition open and closed arms.The interaction effect between the conditions and treated groups was statistically significant(P≤0.0001).Post-hoc analysis using tukeys multiple comparisons test indicated that there was no significant differeance (P≤0.0001) D-gal, ALA treated group between open and closed arm.The main value of Elevated plus-maze test \ open arm was(control,2.6±0.80,D-gal, 8±1.84,ALA,1.6±0.83, ALA+D-gal,7.2±1.01) for groups control, D-gal, ALA and ALA+D-gal.The main value of Elevated plus-maze test \ closed arm was(control, 7.4±0.62, D-gal, 2±0.42, ALA, 8.4±1.20, ALA+D-gal, 2.8±0.78) for groups control, D-gal, ALA and ALA+D-gal. **Figure (4.16) Image(4.4)**

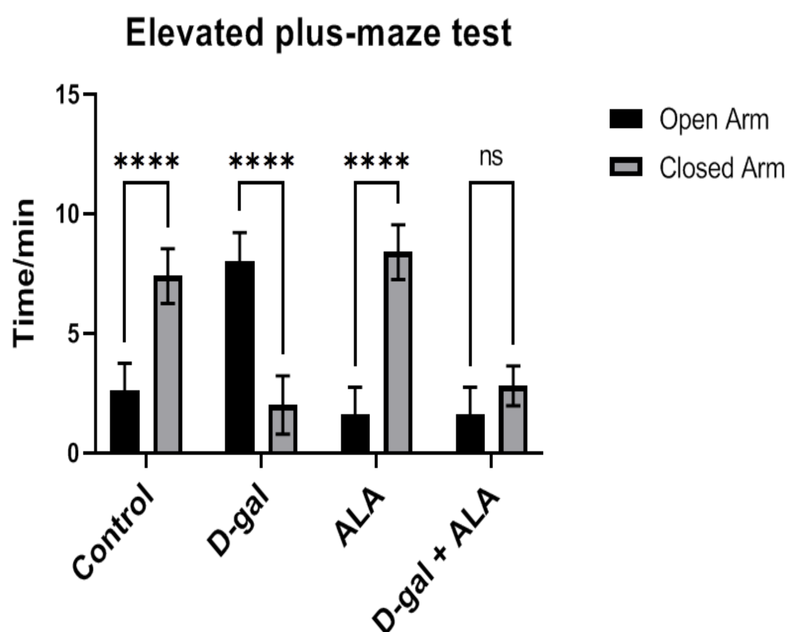

Figure 4.16. Role of ALA on some Elevated plus-maze test \ open& closed arm on brain damage induced by D-gal in male rats. There was a significant difference in

the time of Elevated plus-maze test \ open arm and closed arm in treated groups compare to D-gal group. (****P < 0.0001): values significantly difference from ALA compare to D-gal group. (****P < 0.0001): values was no significantly difference from D-gal + ALA compare to D-gal group. (ns) refers to values significantly non-difference. Data are expressed as mean ± SEM, n= 5

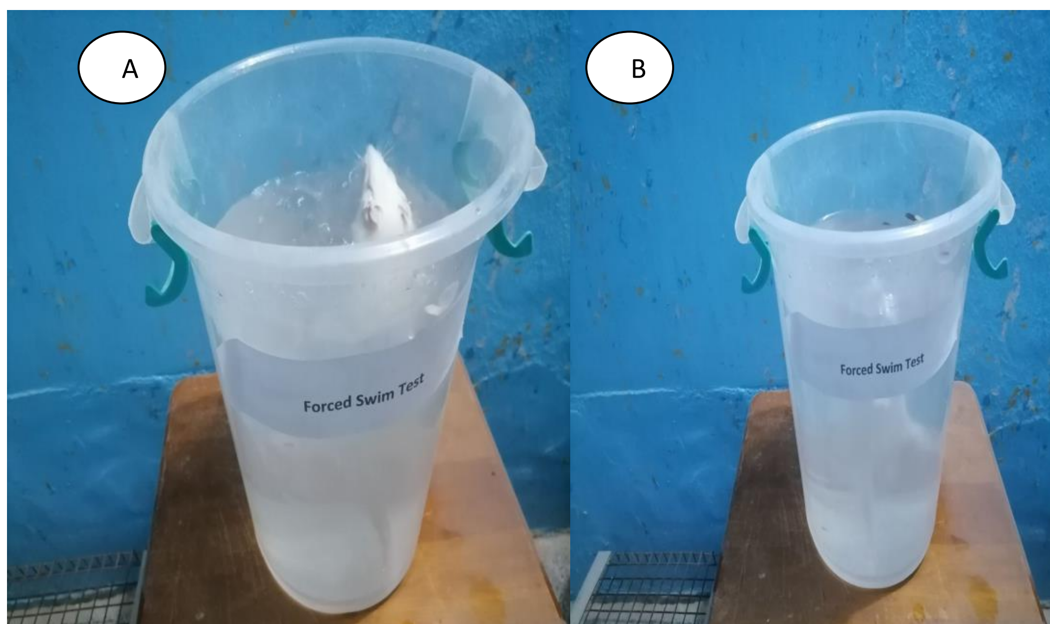

Image 4.1. Forced Swim Test . In these two pictures in the forced swimming test, which measures the period in which the rat can swim for the longest possible period within a period of 15 minutes / we notice that there is a difference between picture **A,** which represents a group of lactose, and picture **B,** which represents a group of control, and we find that there is a difference between the two pictures.

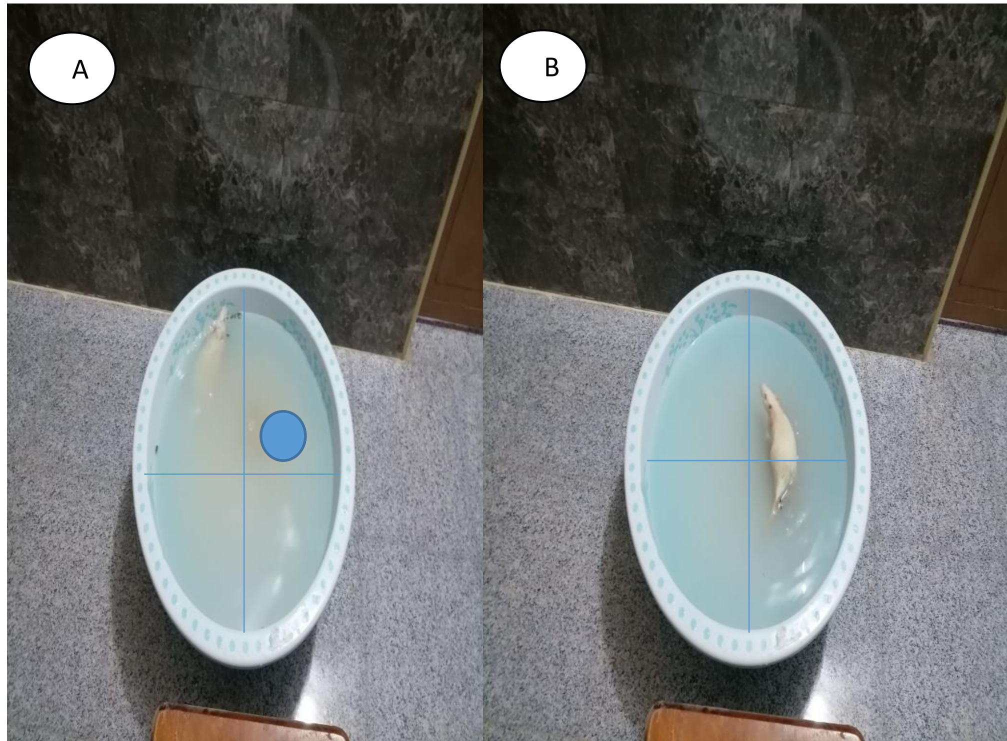

Image 4.2. Morris Water Maze Test . These two pictures are in the Morris maze test, which measures how long a rat can swimAnd return to the platform in the shortest possible period within a period of 90 seconds / we notice that there is a difference between picture **A**, which represents a group of lactose, and picture **B**, which represents a group of control, and we find that there is a difference between the two pictures.

Image 4.3. Dark &Light Test . In these two pictures in the light and dark test, which measures the period that the rat can stay the longest in one of the two roomsduring a period of 5 minutes / we notice that there is a difference between picture **A**, which represents a control group, and picture **B**, which represents a D-galactose group, and we find that there is a difference between the two pictures.

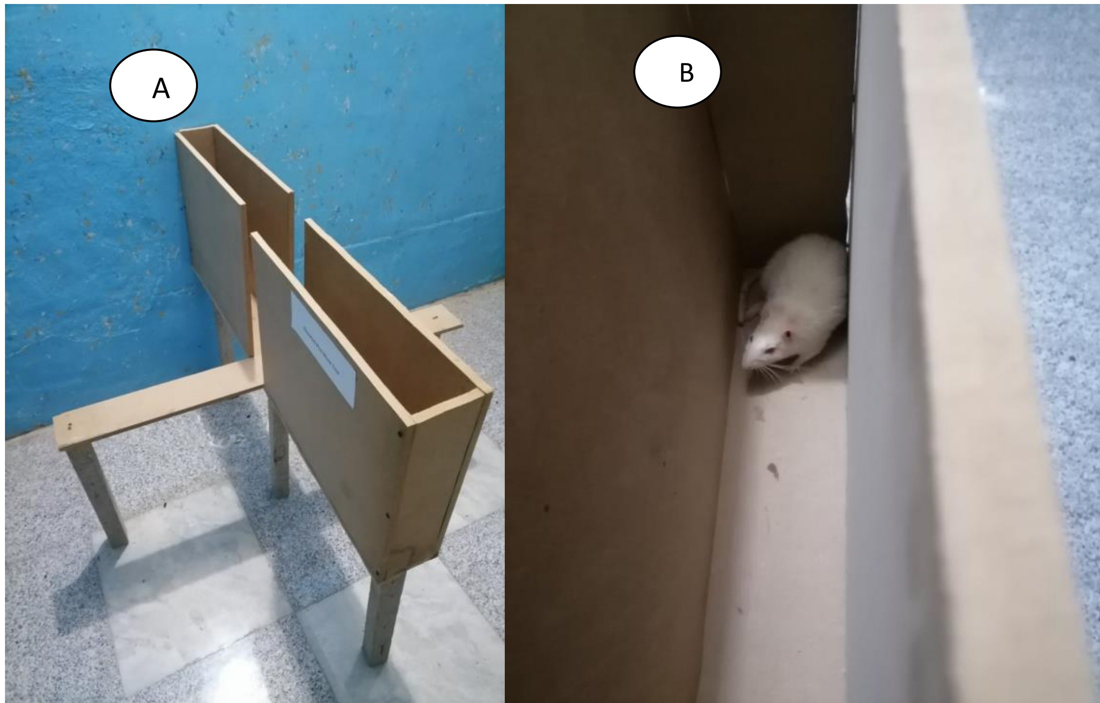

Image 4.4. Elevated Plus Test. The elevated maze test (EPM) is a common behavioral test, most commonly used in the study of fear, anxiety, fear, and anxiety, whether triggered by a painful stimulus, a predator, or even exposure. Bright light produces freezing behavior in many species. In the case of rats, freezing behavior and avoidance of areas with Bright lighting as measures to assess anxiety and fear In behavior tests such as the open field test, the EPM relies on the conflict between the natural tendency of mice and rats to explore a new environment balanced with the medium and anxiety characteristics that result from entering a brightly lit open area and watching there is a difference between the presence of the rat in the closed line And the unclosed line, we find in the control group(**A**) the rat is inside the closed line, while in the D-galactose group(**B**) we find the rat outside to the open line. Within a period of 10 minutes.

5. Discussion

5.1. Role of ALA on some neruometers on brain damage induced by D-gal in male rats:

5.1.1. Role of ALA on the beta amyloid (pg/ml) on brain tissue damage induced by D-gal in male rats

There was a significant increase ($p \le 0.05$) in βA levels in D-gal injected group compared to other group and this agreement with (Hua et al., 2007). Increase in βA result from increase in ROS which cause brain aging and neural degeneration (Liu et al.,2020).

ROS are radicals and molecules deriving from the incomplete reduction of molecular oxygen they are produced in small quantity during the in metabolism of oxygen, through four successive 1-electron reductions of O2 leading to H2O formation they are necessary to maintain the homeostasis in cells and play an important role in sig-naling (Devasagayam et al.,2004).

Free radicals are a sufficient trigger for inducing formation of βA (Thinakaran, &, 2008).That may have major implications as it suggests that mitochondrial dysfunction is involved early in the pathogenesis of sporadic AD fact that mitochon-drial dysfunction associated with enhanced mitochondrial ROS production is consid-ered to be intimately involved in the aging process, βA itself can cause mitochondrial dysfunction and oxidative stress and thereby may start a vicious cycle accelerating amyloid genic Amyloid precursor protein (APP) processing (Leuner et al.,2012).

(APP) is cleaved by the enzyme β-secretase within the extracellular domain, at posi-tion 671, shedding off the soluble ectodomain of APP (sAPP-β) The remaining C-terminal fragment (CTF-β) is subsequently processed by γ-secretase, releasing the βA peptide and the AICD fragment(Thinakaran, & Koo, 2008).

(APP) plays an important role in the generation of βA in the brain The phosphorylation of APP and key enzymes involved in the proteolytic processing of APP has been demonstrated to be critical for modulating the generation of Aβ by either altering the subcellular localization of APP or changing the enzymatic activities of the secretases responsible for APP processing (Zhang et al., 2020).

In the combination group, there was a no significant ($p \geq 0.0001$) decrease in the beta amyloid when compared to the D-gal group because ALA works on reducing in the ROS of brain cell and decrease oxidative stress of nerve cell (Aoyama et al.,2021).

5.1.2. Role of ALA on the serum Acetylcholinesterase (AChE) (U/ml) on brain damage induced by D-gal in male rats

The current study found that there was a significant ($p \leq 0.05$) increase in the Acetylcholinesterase (AChE) in D-gal injected group compared to other groups. And this agreement with (Liaquat et al., 2017; Chiroma et al.,2018) .

Acetylcholinesterase (AChE) activity is increased within and around amyloid plaques, which are present in Alzheimer’s disease (AD) patient’s brain (Melo et al., 2003; Stanciu et al.,2020).Treatment of Alzheimer's disease has been dominated by the use of acetylcholinesterase (AChE) inhibitors,these drugs compensate for the death of cholinergic neurons and offer symptomatic relief by inhibiting acetylcholine (ACh) turnover and restoring synaptic levels of this neurotransmitter, AChE itself has been implicated in the pathogenesis of (AD) In particular, it appears that AChE may directly interact with beta amyloid in a manner that increases the deposition of this peptide into insoluble plaques (Stanciu et al., 2020; Rees & Brimijoin, et al., 2003).

(AChE) in the brains of patients with (AD) has raised much interest of late Despite an overall decrease in the AD brain, the activity of AChE increases around beta-amyloid plaques and indeed, the beta-amyloid peptide can influence AChE lev-els, Such evidence stimulated our interest in the possibility that the levels of AChE and amyloid might vary together in (AD) (García-Ayllón et al.,2008;Majdi et al., 2020).

Cytochemical studies have demonstrated that the AChE associated with senile plaques differs enzymatically from the AChE associated with neurons in several respects Biochemical studies indicated that AChE induces amyloid fibril formation and form highly toxic AChE-Abteta complexes the neurotoxicity induced by AChE-Abeta complexes indicated that they trigger more neurodegeneration than those of the Abeta peptide alone fact that AChE is able to accelerate amyloid formation AChE inhibitors may well provide an attractive possibility for treating Alzheimer's disease (Dinamarca et al .,2010;Jokar, et al.,2020;).

(AChE) increase could be through a high breakdown in the number of neu-rons, which leads to a rise in the free AChE inside the body and this agreement with (Zhang & Greenberg, 2012). In our study, it was found that there was a significant decrease in the level of the AChE in the combination group when compared to D-gal group, and this is likely to the effect of the (ALA), which worked to reduce apoptotic and reduce oxidative stress and that lead to decrease the production of beta-amyloid, and consequently, so AChE is decreased (Majdi et al., 2020).

5.1.3. Role of ALA on the Glutamate in the brain tissue (mol/g) damage induced by D-gal in 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 & Goldin, 2005).

Aols, 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 (Ortega et *al.,* 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 & Sadguna, 1996); Connell *et al.,* 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 & Sadguna *et al.,* 1996).

5.2 Role of ALA on some oxidant-antioxidant on brain damage induced by D-gal in male rats:

5.2.1. Role of ALA on serum Total antioxidant capacity (TAO-C) (U/mL) concentration on brain damage induced by D-gal in male rats

Through our study, it was shown that there is a significant increase in the TAO-C concentration in the glutathione group, while the lowest result was in D-gal group, and this confirms that the D-gal works to reduce the anti-oxidants inside the body, while the combination group was close to the control group and this proves that glutathione played a great role in reducing oxidative stress and scavenger free radicals caused by D-gal and the result agreement with (Al-Kurdy *et al*., 2020).

There are two kinds of antioxidant system, one is enzyme antioxidant system, including superoxide dismutase (SOD),) catalase (CAT), glutathione peroxidase (GSH-Px) the other is non-enzymatic antioxidant systems, including uric acid, vitamin C, vitamin E, glutathione, bilirubin, α-lipoic acid, carotenoid. Antioxidant capacity is thought to be the cumulative effect of all antioxidants in blood and body fluids (Krishnamurthy& Wadhwani, 2012; Moussa *et al.,* 2019).

A variety of antioxidant macromolecules, antioxidant molecules and enzymes in a system can eliminate all kinds of reactive oxygen species and prevent oxidative stress induced by reactive oxygen species (ROS), The total level reflect the total antioxidant capacity in the system (Kurutas *et al.,* 2015).Oxidative stress produced by the high hydrogen peroxide and lipid peroxidation levels and reduced antioxidant enzyme activities (Al-Bazii *et al.,* 2014).

5.2.2. Role of ALA on serum Malondialdehyde (mol/L) concentration on brain damage induced by D-gal in male rats

Present current result shown a significant increase in mad level in D-gal group when compared to other group and this data agreement the present study showed that D-gal increase serum MDA level as result of oxidative stress which is manifested by significant increase in liped peroxidation and reduce in GSH, SOD and CAT (Qu *et al*., 2016) . MDA is evidence of

lipid peroxidtion it is final products of polyunsaturated fatty acids peroxidation in the cells an increase in free radicals causes overproduction of MDA (Kurutas *et al*., 2015).

The present study showed the chronic injection intraprtonaly with D-gal cases oxidative stress which increase level of lipid peroxidation as shown from increase of serum MDA and this agreement with (Hadzi-Petrushev *et al*.,2015.).

Reactive oxygen species readily attack the polyunsaturated fatty acids of the fatty acid membrane, initiating a self-propagating chain reaction the destruction of membrane lipids and the end-products of such lipid peroxidation reactions are especially dangerous for the viability of cells, even tissues enzymatic (catalase, superoxide dismutase) Since lipid peroxidation is a self-propagating chain-reaction, the initial oxidation of only a few lipid molecules can result in significant tissue damage (Mylonas & Kouretas, 1999;Chairuangkitti *et al.,* 2013; Christiansen *et al*., 2015).

Disruption of lipid homeostasis can promote pathological changes that contribute towards biological ageing and age-related diseases several age-related diseases have been associated with altered lipid metabolism and an elevation in highly damaging lipid peroxidation products mitochondrial dysfunction and elevated ROS formation (Ademowo *et al*., (2017) .

(ALA) is an important water-phase antioxidant and essential cofactor for antioxidant enzymes, it provides protection also for the mitochondria against endogenous oxygen radicals. Its high electron-donating capacity combined with its high intracellular concentration endows (ALA) with great reducing power, which is used to regulate a complex thiol-exchange system (Ademowo *et al*., (2017).

5.2.3. Role of ALA on serum Glutathione (GSH) (ug/l) concentration on brain damage induced by D-gal in male rats

Our result show a significant decrease (P≤0.0001) in serum GSH in D-gal group compared to other groups this result is in agreement with (Aquilano *et al*.,2014; Kwon *et al*., 2019). Injection IP of 200 mg /kg, BW of D-gal for 4 weeks induced ROS production in the body could be explained by that D-gal increase the oxidative stress and increase in ROS generation and inhibition of cycteine uptake and production leading to decreased GSH level and increase in ROS (Droge *et al*., 2005).

Alph lipoic acid is an endogen antioxidants reduced in the oxidative status as signalizing of oxidative stress, reflecting the redox commensuration between oxidation and

antioxidation various oxidants and antioxidants have been additive that the administration of D-gal lead to decrease in glutathione (GSH) level in serum (Omidkhoda *et al*.,2020).Significant increase ($P \leq 0.05$) in serum reduced GSH in combination group comparison with D-gal group, this result agree with (Hadzi-Petrushev *et al*.,2015).

ALA is essential factor for mitochondrial energy metabolism, where it plays a key role in defense against respiration-induced reactive oxygen species and in the detoxification of lipid hydroperoxides and electrophiles Moreover, as mitochondria play a central strategic role in the activation and mode of cell death, mitochondrial GSH has been shown to critically regulate the level of sensitization to secondary hits that induce mitochondrial membrane permeabilization and release of proteins confined in the intermembrane space that once in the cytosol engage the molecular machinery of cell death So when delivered exogenous ALA it help in maintenance of oxidative stress and decrease the harmful effect of D-gal (Ribas *et al*.,2014).

5.2.4. Role of ALA on serum catalase (ku/L) concentration and serum Superoxide dismutase (SOD) concentration on brain damage induced by D-gal in male rats :

Present study showed a significant decrease ($P \leq 0.0001$) in CAT & SOD level in group that injected D-gal when compared to other groups an oversupply of D-gal leads to its conversion to D-galacto-hexodialdose and hydrogen peroxide by galactose oxidase and to galactitol by action of aldose reductase (Ho *et al.* 2003). These products can accumulate in cells, which in turn causes osmotic and oxidative stress that may account for the acceleration of aging (Kumar *et al*., *2011*).

SOD is effective in preventing and treating diseases related to superoxide free radicals. When superoxide anion radicals are produced excessively or the SOD concentration is low, excessive superoxide anion will cause oxidation (Younus *et al*., (2018).

CAT is an antioxidant enzyme that mainly exists in erythrocytes and some tissue cells, as well as in mitochondria and the cytoplasm (Selvaratnam & Robaire, 2016). In the process of normal oxidative respiration, organisms constantly produce ROS as a highly active molecule, it contains unpaired electrons the enzymatic system represented by SOD can remove ROS. SOD, as the first line of defense against ROS, mainly disproportionates $O2-$ to $H2O2$ CAT can decompose $H2O2$, produce $H2O2$, and increase the oxygen content in cells (Pawlak *et al*., 1998;Ghosh *et al.,* (2018).

SOD is responsible for the elimination of superoxide anions, the most abundant form of ROS, produced mainly during electron transfer in the mitochondrial respiratory chain (Halliwel *et al.,* 1991).

As a result of this action, hydrogen peroxide is generated and it can be further neutralized by the activity of catalase (CAT) an enzyme that functions as part of the alpha lipoic acid cycle, along with glutathione reductase (GR) However, conflicting results do exist and the association between antioxidant status, lipid peroxidation and aging, seems to display variations depending on the species, strain, sex and examined organs of the experimental animals (Rikans & Hornbrook, 1997).

Also, reported an overproduction of SOD in epidermal cells sensitive to superoxide and hydrogen peroxide, whereas cells with overproduction of CAT were protected against the effects of oxidants (Amstad *et al.*1991).

5.3. Role of ALA on some behavioral analysis on brain damage induced by D-gal in male rats:

5.3.1. Role of ALA on Forced swimming test,(FST) on brain damage induced by D-gal in male rats:

According to this study (Allen & D’Anci et *al.,* (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 *et al.,* 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 &Christians , 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 -ALA 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 &Cossart, *et al.,* 2000).

5.3.2. Role of ALA on Morris Water Maze test, (MWT) on brain damage induced by D-gal in male rats:

D-galactose administration mimics some characters of cognitive dysfunction and oxidative damage;therefore, it is gradually accepted by people and used inage-related disorders like AD, current study, D-galactose senescence mice spent a longer time in finding the hidden platform during the retrieval trial in the Morris water maze test which indicates impairment of memory, Observationhas been further strengthened by EPM test in which Dgalactose showed more latency time to enter into closed arm In our previous report, we found that D-galactose has been reported that rodents injected with D-galactose forproduced memory impairment in rats for 30 days (Dogra & Kumar, 2010).

shows progressive deterioration of learning and memory capacity and increases production of free radicalsin the brain, D-gal administration mimics some characters of cognitive dysfunction and oxidative damage; therefore, it is gradually accepted by people and used in age-related disorders like AD in our study, D-gal senescence mice spent a longer time in finding the hidden platform during the retrieval trial in the Morris water maze test which indicates impairment of memory Observation has been further strengthened by EPM test in which D-gal showed more latency time to enter into closed arm. In current previous report, we found that D-gal produced memory impairment in mice for 4 weeks (Dogra & Kumar, 2010).

Earlier, it has been reported that rodents injected with D-gal for 4–10 weeks shows progressive deterioration of learning and memory capacity and increases production of free radicals in the brain (Song *et al.,* 1999). In our study, chronic administration of D-gal resulted in a marked oxidative stress as indicated by increasing lipid peroxidation, nitrite concentration, and depletion of NP-SH levels, catalase, superoxide dismutase, and glutathione-s-transferase activity, suggesting oxidative damage Afterwards, growing evidence there was learning and memory impairment and neuro-

pathological revealed changes like oxidative damage occurred in the brain of rodents treated with D-gal (Ya-Zhen *et al.,* 2001;, Wei *et al.,* 2005).

5.3.3. Role of ALA on Light& Dark Room test (L&DRT) on brain damage induced by D-gal in male rats:

The light/dark transition test was originally developed by Crawley and colleagues (Crawley & Goodwin, 1980). There are two differences between their original version and our test first, the light chamber is larger than the dark chamber in the original version, whereas the size of the two chambers is the same in our version of the test. Second, in the original er were transparent (Crawley & Goodwin, 1980). while we usversion, the light chamber had no ceiling and the walls of the light chamber opaque white plastic for the ceiling and walls of the light chamber these differences, namely the size and openness of the light chamber, allow for the simultaneous detection of bright-space anxiety as well as open-space anxiety in the original version of the test. In our laboratory, however, open-space anxiety-like behavior in rat is tested in an elevated plus maze he opaque walls and ceiling, size of the light chamber, and our specific light/dark transition test protocol is more specialized for detecting bright-space anxiety compared to the original version (Wei *et al.,* 2005).

5.3.4. Role of ALA on Elevated plus-maze Test/ open& closed arm on brain damage induced by D-gal in male rats

D-gal administration mimics some characters of cognitive dysfunction and oxidative damage; therefore, it is gradually accepted by people and used in age-related disorders like AD in our study, D-gal senescence rats spent a longer time in finding the hidden platform during the retrieval trial in the which indicates impairment of memory Observation has been further strengthened by EPM test in which D-galactose showed more latency time to enter into closed arm. In our previous report, we found that D-gal produced memory impairment in rats for 30 days (Kumar A, Dogra S, *et al.,* 2009;, Kumar A, Prakash A, *et al.,* 2010). Earlier, it has been reported that rodents injected with D-gal for 30 days shows progressive deterioration of learning and memory capacity and increases production of free radicals in the brain group significantly increased as compared to (Song Bao *et al.,* 1999). Elevated Plus Maze. In the present study, D-galactose treatedgroup , Further, D-galactose-treated group showed a significant delayed respectively, as compared to na¨ıve group, demonstrating that chronic D-galactose-induced marked memory impairment. Chronic A LA (100 mg/kg) treatment significantly shortened acquisition latency on day 30 as well as mean retention ransfer latencies to enter close arm on

days as compared to control (D-galactose-treatedgroup) ($P < 0.0001$). However, ALA (100mg/kg) per se treatmentdid show any significant alteration in both acquisition well as retention as compared to group control (Dogra S, *et al.,* 2009).

Chapter six: Conclusions and Recommendations

6.1. Conclusions

From the results obtained from this study, it could be concluded that:

A- Intraperitoneal injection of 200mg/kg B.W. D-gal for 30 days for male rats caused oxidative stress and significant elevation in the serum MDA, and ACHE activity and a significant decrease in serum concentration, CAT, and SOD activities.

Also the current study illustrated a significant increase in the beta-amyloid and glutamate concentration in the brain tissue inflammation and accumulation of beta amyloid and Glutamate.

B- Ameliorate effect of 100 mg/kg BW daily for 30 days of ALA caused a significant decrease in the beta amyloid and glutamate concentration in the brain tissue.

C- Ameliorate effect of ALA on the behavioral test (FST and LDT) and memory (MWM and EPM) in the ALA and D-gal + ALA compare with D-gal and control groups

6.2. Recommendations

1-Future study about mitochondrial in the central nerves system.

2-Studing the effect of exogenous antioxidant and their effect on the nerves tissue aging in rat.

3- Further studies on the biomarker of aging and their relation to Alzheimer disease.

4 – In Vivo and in vitro studies about brain effected of aging in the BBB.

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Appendices

Appendices I

Measurement of Tissue Beta amyloid:

Test principle

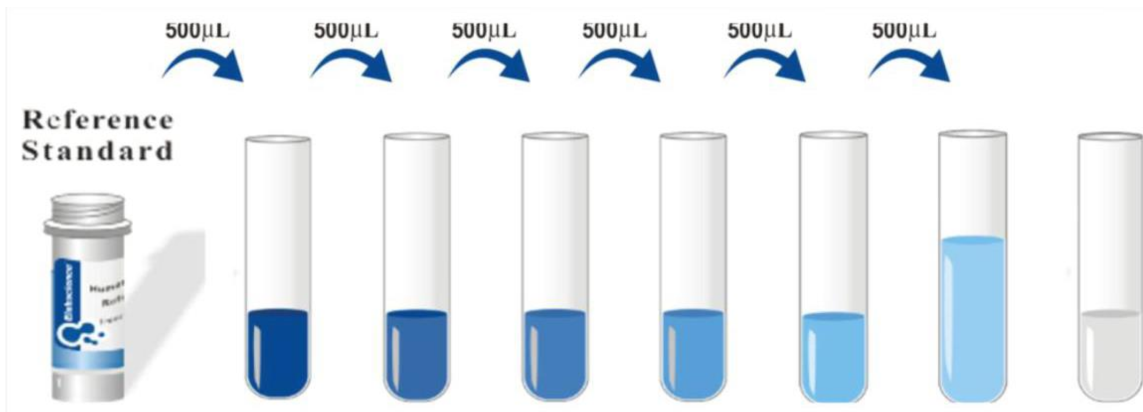
This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Rat Aβ1-42. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Rat Aβ1-42 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Rat Aβ1-42, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Rat Aβ1-42. You can calculate the concentration of Rat Aβ1-42 in the samples by comparing the OD of the samples to the standard curve.

1-Reagent preparation

1. **Wash Buffer:** Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer.

2. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10 min and invert it gently several times. The recommended dilution gradient is as follows: 1000, 500, 250, 125, 62.5, 31.25, 15.63, 0 pg/mL. Dilution method: Take 7 EP tubes, add 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500uL of the 1000 pg/mL working solution to the first tube and mix up to produce a 500 pg/mL working solution. Pipette 500uL of the solution from the former tube into the latter one according to these steps. The illustration below is for reference.

3. **Biotinylated Detection Ab working solution:** Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1×working solution with Biotinylated Detection Ab Diluent.



4. **Concentrated HRP Conjugate working solution:** Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Dilute the 100× Concentrated HRP Conjugate to 1× working solution with Concentrated HRP Conjugate Diluent.

2- Assay procedure

1. Add 100 µL standard or sample to each well. Incubate for 90 min at 37°C.
2. Remove the liquid. Add 100 µL Biotinylated Detection Ab. Incubate for 1 hour at 37°C.
3. Aspirate and wash 3 times.
4. Add 100 µL HRP Conjugate. Incubate for 30 min at 37°C.
5. Aspirate and wash 5 times.
6. Add 90 µL Substrate Reagent. Incubate for 15 min at 37°C.
7. Add 50 µL Stop Solution. Read at 450 nm immediately.

3-Calculation of results

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis. If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

Appendices II

Measurement of serum Acetylcholine Esterase

Test principle

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Rat AChE. Samples (or Standards) are added to the micro

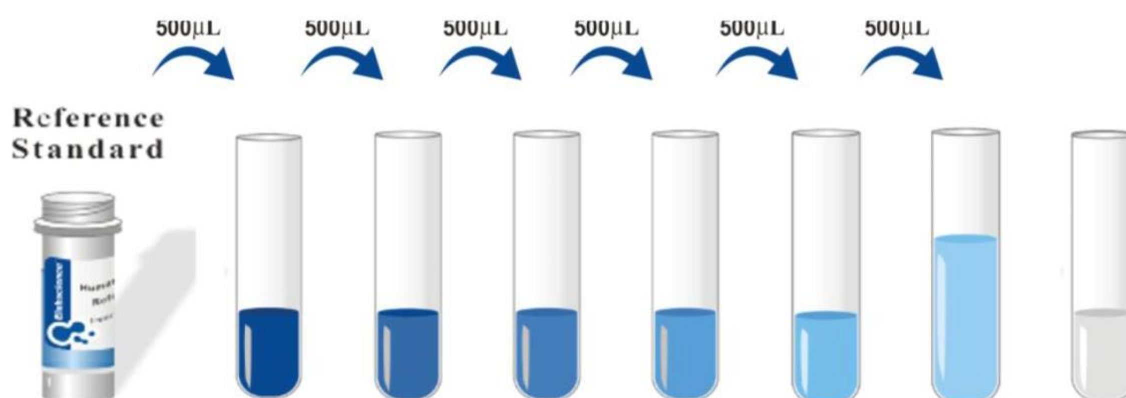
ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Rat AChE and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Rat AChE, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

1 -Reagent preparation

1. Bring all reagents to room temperature (18~25℃) before use. Follow the Microplate reader manual for set-up and
preheat it for 15 min before OD measurement.

2. **Wash Buffer:** Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare
750 mL of Wash Buffer.Note: if crystals have formed in the concentrate, warm it in a 40℃ water bath and mix it gently until
the crystals have completely dissolved

3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 50 ng/mL. Then make serial dilutions as needed.
The recommended dilution gradient is as follows: 50, 25, 12.5, 6.25, 3.13, 1.57, 0.78, 0 ng/mL.



Dilution method: Take 7 EP tubes, add 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500uLof the 50 ng/mL working solution to the first tube and mix up to produce a 25 ng/mL working solution. Pipette 500uL

4. **Biotinylated Detection Ab working solution:** Calculate the required amount before the experiment (100 μL/well).

In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1×working solution with Biotinylated Detection Ab Diluent.

5. **Concentrated HRP Conjugate working solution:** Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Dilute the 100× Concentrated HRP.

2 Assay procedure:

1. Add the Standard working solution to the first two columns, Add the samples to the other wells (100 uL for eachwell). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃.

2. Remove the liquid out of each well, do not wash. Immediately add 100 μL of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer. Gently mix up. Incubate for 1 hour at 37°C.

3. Aspirate or decant the solution from each well, add 350 uL of wash buffer to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps.

4. Add 100 μL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.

5. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 3.

6. Add 90 μL of Substrate Reagent to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light.

7. Add 50 μL of Stop Solution to each well

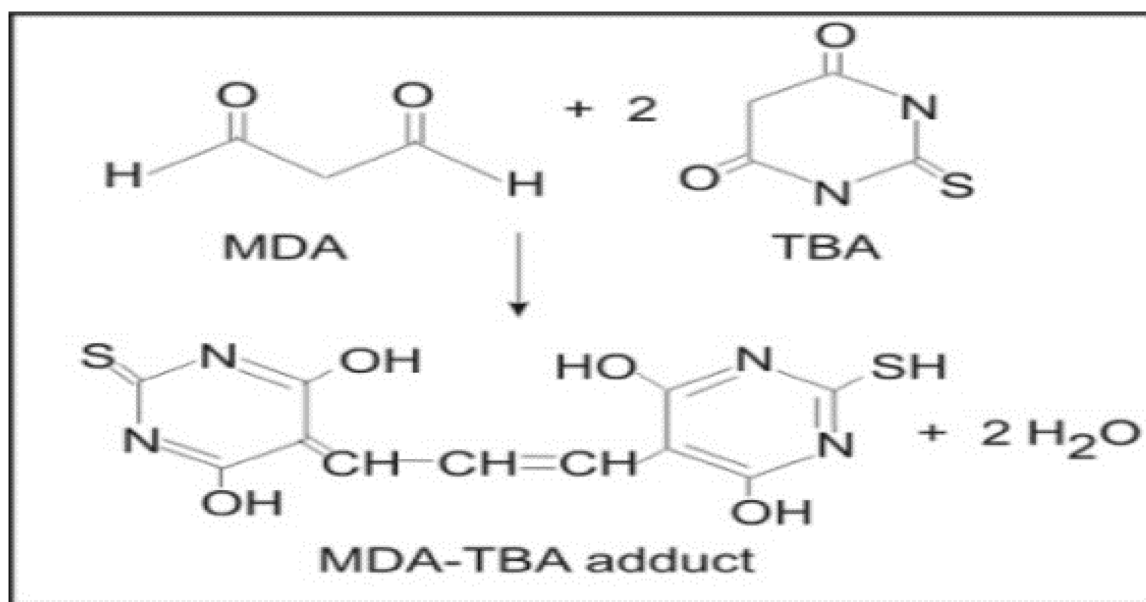
8. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Appendices III

Determination of Serum Malondialdehyde:

Principle:

This method quantifies lipid peroxides by measuring aldehyde breakdown products of lipid peroxidation. Basic principle of the method is the reaction of one molecule of malondialdehyde and two molecules of thiobarbituric acid to form a red MDA-TBA complex which can be measure at 535 nm.



Stock TCA – TBA – HCl Reagent:

It was prepared by dissolving 15% W/V trichloroacetic acid and 0.375% W/V thiobarbituric acid and 0.25N HCl to make 100 ml (2.1 ml of concentrated HCl in 100 ml). This solution was mildly heated to assist in the dissolution of TBA. Dissolved 15 gm TCA and 0.375 mg thiobarbituric acid in 0.25 N HCl and volume was made up to 100 ml with 0.25 N HCl.

Procedure:

To 0.4 ml of serum, 0.6 ml TCA-TBA-HCl reagents were added. It was mixed well and kept in boiling water bath for 10 minutes. After cooling 1.0 ml freshly prepared 1N NaOH solution was added to eliminate centrifugation. This absorbance of pink colour was measured at 535 nm

against blank which contained distilled water in place of serum. In blank 0.4 ml distilled water and 0.6 ml TCA-TBA-HCl reagent was mixed and boiled. Blank was always taken.

Calculation:

extinction coefficient of MDA at 535 nm is = 1.56 × 105

MDA concentration = χ / 0.0624 nmol / ml

Appendices IV

Determination of serum catalase concentration:

Procedure:

Catalase activity was assessed by incubating the enzymes ample in 1.0 ml substrate (65 mmol/ml hydrogen peroxide in 60 mmol/l sodium–potassium phosphatebuffer,pH7.4)at37 °C for three minutes. There action was stopped with ammonium molybdate. Absorbance of the yellow complex of molybdate and hydrogen peroxide is measured at374nm against the blank.

Reagents

1. Sodium, potassium phosphate buffer (50mM,pH7.4): this buffer isprepared by dissolving 1.1g of Na2HPO4 and 0.27g of KH2PO4 in 100ml distilled water.
2. H2O2 (20 mM) in 50mmol/l sodium, potassium phosphate buffer: this solution is freshly diluted and standardized daily using a molar extinction coefficient of 43.6M_1 cm_1 at 240nm.

3. Ammonium molybdate (32.4mmol/l).

Table 1

Reagents	Test	Control-test*	Standard	Blank
Serum	100 μl	100 μl	-	-
D.W.	-	1000 μl	100 μl	1100 μl
Hydrogen peroxide	1000 μl	-	1000 μl	-
Mix with vortex and incubate at 37 °C for 3 min, after that, add:				
Ammonium molybdate	4000 μl	4000 μl	4000 μl	4000 μl
After that, the tubes were kept at room temperature. Changes in absorbance were recorded at 374 nm against the reagent blank.				

4. Calculation The rate constant of a first-order reaction (k) equation is used to determine catalase activity:

t: time.

S°: absorbance of standard tube

S: absorbance of test tube.

M: absorbance of control test (correction factor).

Vt: total volume of reagents in test tube. Vs: volume of serum.

Appendices VII

Determination of serum concentration of superoxide dismutase (SOD) activity determination:

Preparation

1. Tris buffer (pH 8.0): was prepared by dissolving 0.258 gm of tris and 0.111 gm of Ethylenediaminetetraacetic acid (EDTA) in dH2O and completing the volume to 100 ml.

2. Pyragallol solution (0.2 mM): was prepared by dissolving 0.0252 gm of pyragallol with 10 ml of HCl and completing the volume to 100 ml with dH2O.

Procedure

According to Marklund and Marklund (1974), reaction mix is consisting of 50 µl crude enzyme extract with 2 ml of tris buffer and 0.5 ml of pyragallol (0.2 mM) which absorbs light at 420 nm. Control solution contains the same materials except for the enzyme extract that was replaced by dH2O. As a blank, dH2O was used. Single unit of enzyme is defined as the amount of enzyme

that is capable of inhibiting 50% of pyragallol oxidation. SOD activity was calculated using the following equation (Ma *et al.*, 2009) :

SOD activity (u/ml)= (Vp-Vs)/(Vp*0.5)*(Vt/Vs)*n

Vp=Auto oxidation rate of pyrogallol rate of pyrogallol (control)

Vs= Auto oxidation rate of sample (with enzyme)

Vt=Total reaction volume (ml)

Vs= volume of enzyme used for the assay (ml)

n= dilution fold of the SOD sample

0.5= factor for 50% inhibition

Appendices VIII

Determination of serum Total Antioxidant Capacity (T-AOC):

Preparation:

1. Preparation of reagent 2 working solution:

Dissolve a vial of reagent 2 with 120 mL double distilled water fully (It can be Dissolved by incubating in 80-90℃ water bath). It can be used after cooling to room temperature.

2. Preparation of reagnt 3 working solution:

Dilute the reagent 3 with reagent 4 at the ratio of 1:19. Prepared the working Solution before use.

Procedure

(1) Sample tube: Add 1.0 mL of reagent 1 to 5 mL EP tube.

Control tube: Add 1.0 mL of reagent 1 to 5 mL EP tube.

(2) Sample tube: Add A* mL of sample to the tube.

Control tube: Add nothing.

(3) Add 2.0 mL of reagent 2 working solution and 0.5 mL of reagent 3 working solution to each tube.

(4) Mix fully and incubate the tubes at 37℃ for 30 min.

(5) Add 0.1 mL of reagent 5 to each tube.

(6) Sample tube: Add nothing.

Control tube: Add A* mL of sample to the tube.

(7) Mix fully and stand for 10 min at room temperature. Set to zero with double distilled Water and measure the OD value of each tube at 520 nm with 1 cm optical path quartz Cuvette.

Calculation

Use this equation to calculate the t-aoc

t-aoc =$\triangle A 0.01 \div 30 \times v1 v2$

ΔA: ODSample – ODControl

*: The reaction time, 30 min.

V1: The total volume of reaction, mL.

V2: The volume of sample added to the reaction, mL .

centrifuged at 4500 × g for 10 min. 1.0 mL of supernatant was added to 4.0 mL of 0.3 M Na2HPO4 solution and 0.5 mM DTNB (5,5 -dithiobis-2-nitrobenzoic acid) was then added to this solution. Reduced glutathione was measured as the difference in the absorbance values of samples in the presence and the absence of DTNB at 412 nm. GSH value was calculated as nmol GSH/mg protein in the tissues and mmol GSH/g Hb in whole blood using the reduced glutathione as a reference (hemoglobin levels were estimated in whole blood using the Drabkins' solution).

Appendices IX

Determination of Morris water maze Test (MWM).

Protocol:

A spatial memory test was performed. The Morris water maze is a white circular pool (diameter: 150 cm and height: 35 cm) with a featureless inner surface circular pool was filled with nontoxic water and kept at 23-25 °C. The pool was divided into four quadrants of equal area. A transparent plastic platform (4.5 cm in diameter and 14.5 cm in height) was centered in one of the four quadrants of the pool. There are four prominent visual cues on each side of four quadrants of the pool. The swimming route of rats, from the start position to the platform, and the platform was submerged

0.5-1.0 cm below the water surface so that it was invisible at water level. trial, the rats was placed into the water maze at one of three randomly determined locations and released allowing

the animal to find the hidden platform. After the rats found and climbed onto the platform, the trial was stopped and the escape latency was

recorded. The maximum trial length was 90 s. If animals did not locate the platform within 90 s, the experimenter guided the mouse by hand to the platform, then the rats was kept on the escape platform for 30 s and an escape latency of 90 s was recorded. order to assess the spatial retention of the location of the hidden platform, was conducted 24 h after the last acquisition session. During this trial, the platform was removed from the maze, and each mouse was allowed to search the pool for 90 s before being removed. The time spent in the target quadrant was used as a measure of consolidated spatial memory.

Appendices X

Determination of Forced Swimming Test (FST).

Protocol:

Forced swim test (FST)The studies were carried out on rats according to themethod of (Porsolt *et al.* 1977). rat were placed indi-vidually into glass cylinders (height 25 cm, diameter10 cm) containing 10 cm of water, maintained at 23–25°C.The animals were left in the cylinder for 15 min. the total duration of immobility was with a summing stopwatch. The mousewas judged to be immobile when it remained floatingpassively in the water, performing slow motion to keephead above the water

Determination of Light/Dark Test (LDT):

Protocol:

1.The apparatus used for the light/dark transition test consisted of a cage (21x42x25 cm) divided into two sections of equal size by a partition with door (Ohara & Co., Tokyo).

2.Rat are housed three to five per cage in a room with a 12 hr light/dark cycle . All the cages containing mice are transferred to the behavior testing room 30 min before the trial begins.

3.One chamber is brightly illuminated , whereas the other chamber is dark , Rat are placed into the dark side and the door is opened automatically 3 seconds after the mouse is detected , The door is used so that the mice do not enter the light chamber immediately after the release with their motivation to escape from experimenter, since the latency to enter the light chamber may serve as an index of anxiety-like behavior.

4.Rat are allowed to move freely between the two chambers with door open for 5 min. (Crawley J, N. *et al.* 1985).

5.The in each chamb the time spent in the each chamber,

6.After each trial, all chambers are cleaned with super hypochlorous water to prevent a bias based on olfactory cues.

Appendices XI

Determination of Elevated Plus-Maze Test (EPM).

Protocol:

Elevated plus maze from rats were tested on the elevated plus maze . The black polypropylene maze was elevated 1 M from the ground with four 10.cm wide×50.cm long runways (Med-Associates, St Albans, VT, USA). The two closed runways had 40. cm black polypropylene walls. To begin the test, rats were placed at the center of maze facing a closed arm. Plus-maze behavior was measured as the time spent of the maze during a 10 min. session as determined by the average of counts recorded by an automated system and by an observer blind to the experimental treatment condition. For subjective rating, entries were recorded when a rat had four paws on an arm and explorations were recorded when a rat had two paws on an arm. Time spent on the open arms was defined as the sum of the intervals between open and closed arm entries.

Protocol:

1.The apparatus used for the light/dark transition test consisted of a cage (21x42x25 cm) divided into two sections of equal size by a partition with door (Ohara & Co., Tokyo).

2.Rat are housed three to five per cage in a room with a 12 hr light/dark cycle . All the cages containing mice are transferred to the behavior testing room 30 min before the trial begins.

3.One chamber is brightly illuminated , whereas the other chamber is dark , Rat are placed into the dark side and the door is opened automatically 3 seconds after the mouse is detected , The door is used so that the mice do not enter the light chamber immediately after the release with their motivation to escape from experimenter, since the latency to enter the light chamber may serve as an index of anxiety-like behavior.

4.Rat are allowed to move freely between the two chambers with door open for 5 min. (Crawley J, N. et al. 1985).

5.The in each chamb the time spent in the each chamber,

6.After each trial, all chambers are cleaned with super hypochlorous water to prevent a bias based on olfactory cues.

Appendices XII

Determination of tissue Glutamate.

Sample collection

1. The tube for blood collection should be free of pyrogen and endotoxin
2. Hemolysis and hyperlipidemia specimens can not be used to extracted serum and plasma.
3. The samples should appear clear and transparent. And all the suspension should be removed through centrifugation.
4. If collected samples are not timely detected, they should be divided according to single usage amount and frozen reserved in refrigerator at -20-80℃, avoiding the repeated freeze-thaw.
5. According to the actual situation of the samples, make proper multiple dilutions (Pre-experiment is strongly recommended in order to confirm the dilution ratio)
6. Collect specimens and try to gain double dosage to avoid specimens shortage for repeated assays in case that failure in one-assay delays experimental process.
7. Do protective measures when collecting specimens (e.g. wearing gloves, respirator, respirator, etc.), aware of the potential risk in all specimens.
8. Specimen processing should be inside the biological safety cabinet. Ensure proper use of the biological safety cabinet.

Measures for the samples

1. Serum: Put the collected whole blood in refrigerator at 4℃ for the night. Then centrifuge it for 10min at 1000-3000rpm. Take supernatant tested immediately or put samples at -20℃(for 1-3 months) or -80℃ (for 1-3 months) for storage.
2. Plasma: Take EDTA, sodium citrate and heparin as anticoagulant. Add the plasma and mix them well. Centrifuge mixture for 10min at 1000-3000rpm. Take supernatant tested immediately or put samples at -20℃(for 1-3 months) or -80℃ (for 1-3 months) for storage.
3. Tissue homogenate: Take tissue slices and wash them out in 0.01MPBS; Add tissue protein extraction reagent according to proportion of 1G: 5-10ml and mix them in ice

water. After being blended, mixture shall be centrifuged for 10min at 5000-10000rpm.Take supernatant tested immediately or put them at -20℃(for 1-3 months) or -80℃ (for 1-3 months) for storage.

4. Cell culture: Take centrifugation for 10min at 1000-3000rpm.Take supernatant tested immediately or put samples at -20℃(for 1-3 months) or -80℃ (for 1-3 months) for storage.

5. For urine, ascites, cerebrospinal fluid, etc: ake centrifugation for 10min at 1000-3000rpm. Take supernatant tested immediately or put samples at -20℃(for 1-3 months) or -80℃ (for 1-3 months) for storage.

Note: The general principles of the sample dilution

The user should refer to the references to know the probable content of the samples before decide to dilute the samples,and the diluted content of the sample must be in the best detection range of the given ELISA Kits. The dilution of the sample should be recorded in detail.

【Note】

1. The kit should be kept at 2-8℃ before being used. Except the redissolved standard samples, other Ingredients must not be frozen.
2. For the concentrated biotinylated Rat Glu antibody, the concentrated enzyme-conjugates have small size. Bumping or potential inversion of the tubes during transportation may cause the liquid sticking to wall or cap. Thus, the tubes should be shaken manually or centrifuged for 1 min at 1000rpm to shake off the adherent liquid down to the tube bottom.
3. Concentrated washing buffer may crystallize a little. Use water bath to help the dissolution during diluting process. The crystals must be totally dissolved when preparing washing buffer.
4. During testing process, the Rat Glu lyophilized standard sample shall be single-use and must not be divided. The sample will quickly inactivate after being dissolved because of its lower concentration.
5. Operation should be strictly in accordance with the instructions. Mixed usage of components with different batch number in this reagent is not allowed
6. Ensure the reagent well mixed by the spiral hybrid instrument. For the reagent in the

microplate, adequate mixing is particularly important to test result. So it's better to employ the micro-oscillator (at the lowest frequency). If there is not micro-oscillator, shake the microplate manually for 1 min, slightly as like a circular movement to make sure reaction liquid in microplate well mixed

7. ELISA for experiment should be strictly operated according to manual standard and fully preheated beforehand.

8. During enzyme immunoassay, there should be multiple pores when testing Rat Glu standard samples

9. Put the unused microplates into raw foil bag at 2-8℃ for storage.

10. Chromogen reagent is sensitive to light. Therefore it should be free of being exposed to light.

11. Kits out of validity should not be used in experiment.

12. The determination of test results must be subject to ELISA's readings. When using dual-wavelength for test, the wavelength should be set at 450nm and 630nm respectively.

13. All the samples, washing liquid and wastes should be treated as biowaste. Colour Reagent C should be 1M sulfuric acid and pay attention to safety when it is used.

14. Sample-adding at every step should be taken by adding instrument. Calibrate accuracy of the adding device to avoid experiment error. The time of single sample-adding should be controlled within 5 min. Just in case of exceeding samples, the volley for sample-adding is proposed.

15. Adhesive closures do not reuse or according to the experiments need to be cropped. Stick a strip of adhesive to compaction

16. Test determination and standard curve should be made at same time in every experiment, so there better be multiple pores. If the content of test sample were too high (OD value of the sample is higher than that of sample well maximum concentration), dilute to certain multiple by sample diluents (n times), then test the result and multiply it by dilution ratios when making calculation.

17. The sample containing NaN3 can't be tested because NaN3 inhibit the activities of horseradish peroxidase (HRP).

18. When washing board by plate washer, the volume of liquid injection to each well should be more than 350µl. Check if the sampling head is jammed. Yet the water absorption

material with Paper Scrap should be cautiously used while washing board manually, free of the reaction between exogenous peroxidase analogues and chromogen reagents.
19. After the reaction being terminated by Colour Reagent C, read OD within 10 min.
20. During multiple pores experiment, the calculation result shall be mean value.
21. Sample hemolysis may cause false positive result, so this test is not appropriate for sample hemolysis.
22. During test, the strips should be put into closed box after adding samples and the humidity around should be kept at about 60%
23. It is advisable to check the thermostat by frequent calibration to keep its inside temperature at 37℃. Ensure the experimental temperature being steady.
24. For 48T Elisa Kit, all components are 50% amount of 96T.
25. If there is any difference, the English instruction shall prevail.

Test preparation

1.Please get the Elisa Kit out of refrigerator 20 minutes in advance and take test when it balances to room temperature.

2. Dilute the concentrated washing solution with double distilled water (1:25). Put the unused back.

3. Rat Glu standard sample: Add diluent 1.0ml into Rat Glu lyophilized standard sample and keep it still for 30 min. After the sample completely dissolved, mix it slightly and mark label on the tube①, then take dilution as needed.(It is recommended to using following concentration value to standard curve: 20,10, 5, 2.5, 1.25, 0.625, 0.312nmol/ml). Note: Make sure the lyophilized standard completely dissolved and well mixed.

4. Legend of standard sample dilution method: Take 7 clean tubes and label them with ②,③,④,⑤,⑥,⑦,⑧ respectively. Add 300μl standard sample diluent into each tube.

Pipette out 300μl diluent from tube ① to tube ② and mix well. Further Pipette out 300μl diluent from tube ② to tube ③, and mix well. Repeat steps above up to tube ⑦. Standard sample dilution in tube⑧is negative control.

Note: The redissolved standard liquid（20 nmol/ml）shall be discarded and not non-reusable.

Note: Reconstituted standard stock solution can not be reused.

5. Biotinylated Rat Glu antibody liquid: Referring to needed amount, employ antibody diluent to dilute the concentrated biotinylated antibody (1:100) to form biotinylated antibody liquid. The preparation should be done 30 min in advance. And it′s only for use on that day

6. Enzyme-conjugate liquid: Referring to needed amount, dilute the concentrated enzyme-conjugate by enzyme-conjugate diluent (1:100) to form enzyme-conjugate liquid. The preparation should be done 30 min in advance. And it′s only for use on that day.

7. Colour Reagent liquid: Prepare Colour Reagent liquid 30 min in advance with Colour Reagent A and Colour Reagent B by the proportion of 9:1.

Washing method

1.Automatic plate-washing machine: The required amount of lotion is 350μl and the injection and extraction interval should be 20一30secs. Be well aware of the operation instruction before putting the machine into practice.

2. Manual plate-washing machine: add 350μl lotion to each well and keep it still for 30secs.

Shake individual wells as dry as you can and clean them with absorbent paper. During the plate-washing process, pay attention to the lotion-adding step to avoid contamination and well-jumping..

【Steps】

1.Take out needed strips from zip lock bag which balances to room temperature. The unused strips and desiccant should be put back into the sealed aluminum foil bag at 2-8℃ for storage.

2. Set aside blank wells (if dual-wavelength reading plate is used, the blank wells could be ignored)

3. Add samples or different concentration of Rat Glu standard samples to corresponding wells (100μl for each well), 0nmol/ml well should be filled with standard diluent. Seal the reaction wells with adhesive tapes, hatching in incubator at 37℃ for 90 min.

4. Prepare biotinylated Rat Glu antibody liquid 30min in advance.

5. Wash the Elisa plate 2 times

6. Add the biotinylated Rat Glu antibody liquid to each well (100μl for each). Seal reaction wells with adhesive tapes, hatching in incubator at 37℃ for 60 min.

7. Prepare enzyme-conjugate liquid 30min in advance.

8. Wash the Elisa plate 3 times

9. Add enzyme-conjugate liquid to each well except blank wells (100μl for each). Seal the reaction wells with adhesive tapes, hatching in incubator at 37℃ for 30 min.

10. Wash the Elisa plate 5 times.

11. Add 100μl Colour Reagent liquid to individual well (also into blank well), hatching in dark incubator at 37℃. When color for high concentration of standard curve become darker and color gradient appears, the hatching can be stopped. The chromogenic reaction should be controlled within 30 min.

12. Add 100μl Colour Reagent C to individual well (also into blank well). Mix well. Read OD（450nm） within 10 min.

Note:It doesn′t need to add any reagents in blank well except Color reagent solution and Color reagent C.

الملخص

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أجريت هذه الدراسة لتقيم الدور الوقائي لحمض ألفا ليبويك (ALA) على تلف الجهاز العصبي المركزي الناجم عن فرط D-galactose في ذكور الجرذان.

تم تقسيم أربعين من ذكور الجرذان البالغة إلى أربع مجموعات (10 / مجموعة) ، وحقنت المجموعة الأولى بمحلول ملحي طبيعي لمدة ثلاثين (30) يومًا وعملت كمجموعة ضابطة (مجموعة السيطرة). تم حقن الجرذان في المجموعة الثانية بـ 200 مجم / كجم من وزن الجسم لمدة 30 يوم (D-gal) ، وحقنت جرذان المجموعة الثالثة (ALA) بـ (IP) (D-gal) بنسبة 100 مجم / كجم. B .W لمدة 30 يومًا ، والمجموعة الرابعة (D-gal + ALA) تم حقنها بـ D-galactose و D-gal مع 200 مجم / كجم من وزن الجسم مع 100 مجم / كجم من وزن الجسم. يوميا لمدة 30 يوما. نفذت التجربة في مختبرات كلية الطب البيطري جامعة كربلاء.

تم جمع عينات الدم أثناء الصيام باستخدام تقنية البزل القلبي بعد ثلاثين (30) يومًا من التجارب لجمع أنسجة المخ لقياس: نشاط بيتا أميلويد ، جلوتامات ، مصل أستيل كولين استريز (AChE) ، مجموع مضادات الأكسدة Capaci-ty (TAC)، Malondialdehyde (MDA) ، تركيز الجلوتاثيون المنخفض (GSH) ، أنشطة كاتا ليز (CAT) ، ديسموتاز الفائق (SOD) وتقييم التحليل السلوكي ، اختبار السباحة القسري (FST) ، اختبار الضوء / الظلام (LDT) واختبارات الذاكرة اختبار متاهة موريس المائية (MWM) ، اختبار المتاهة الإضافية المرتفعة (EPM).

أظهرت النتائج زيادة معنوية (P≤0.0001) في قيمة بيتا أميلويد أنسجة المخ في المجموعة المعالجة D-gal مقارنة بمجموعة التحكم ، ALA و (D-gal + ALA). كان هناك انخفاض معنوي (P≤0.0001) في تركيز الغلوتامات في الأنسجة في المجموعة المعالجة بـ ALA بالمقارنة مع مجموعة التحكم ، ALA و (D-gal + ALA). بينما ، لم تكن هناك تغييرات معنوية (P≥0.0001) بين مجموعات التحكم و ALA و (D-gal + ALA). بالإضافة إلى ذلك ، أظهرت النتيجة انخفاضًا معنويًا في تركيز TAO في مصل الدم (P≤0.0001) في المجموعة المعالجة D-gal بالمقارنة مع مجموعة التحكم ، ALA و (D-gal + ALA) ، بينما لم يكن هناك معنوية (P≥. 0.0001) بين مجموعات التحكم و ALA و (D-gal + ALA). يُظهر تركيز GSH في المصل انخفاضًا كبيرًا (P≤0.0001) في مجموعة D-gal بالمقارنة مع مجموعات التحكم و ALA و (D-gal + ALA). لم يكن هناك معنوي (P≥0.0001) بين مجموعات التحكم و ALA و (D-gal + ALA).

أظهرت الدراسة الحالية زيادة معنوية في تركيز MDA (P≤0.0001) في مجموعة D-gal بالمقارنة مع مجموعة التحكم و ALA و (D-gal + ALA). لم يكن هناك تغير كبير (D-gal (P≥0.0001 بين مجموعات التحكم و ALA و (D-gal + ALA). بينما أظهر نشاط المصل (CAT) انخفاضًا معنويًا (D-gal (P≤0.0001 بالمقارنة مع مجموعة التحكم ، ALA و (D-gal + ALA). لم يكن هناك تغيير معنوي لمجموعة ALA (P≥0.0001) بين مجموعات التحكم و ALA و (D-gal + ALA). أظهر تركيز المصل (SOD) انخفاضًا معنويًا (P≤0.0001) في مجموعة D-gal عند مقارنته مع مجموعة التحكم ،

مجموعات ALA و (D-gal + ALA). لم تكن هناك تغييرات كبيرة (D-gal (P≥0.0001 بين مجموعة التحكم و ALA و (D-gal + ALA).

أظهر التحليل السلوكي واختبار الذاكرة انخفاضًا كبيرًا في FST (p≤0.0001) في مجموعة D-gal عند مقارنتها بمجموعات التحكم و ALA و (D-gal + ALA). لم يكن هناك D-gal معنوي (p≥0.0001) بين مجموعات التحكم و ALA و (D-gal + ALA). بينما أظهر زيادة معنوية (p≤0.0001) في (MWM) في مجموعة D-gal بالمقارنة مع مجموعة التحكم ، ALA و (D-gal + ALA) ، ولم تكن هناك تغييرات معنوية (p≥0.0001) في D- gal بين مجموعات التحكم و ALA و (D-gal + ALA). أظهر اختبار Light Room زيادة معنوية (P≤0.0001) في مجموعة D-gal المعالجة عند مقارنتها بمجموعة التحكم ، ALA و (D-gal + ALA) ، كما لم يكن هناك تغير كبير (D-gal (P≥0.000 بين مجموعات التحكم و ALA و (D-gal + ALA). يُظهر اختبار الغرفة المظلمة انخفاضًا كبيرًا (P≤0.05) في المجموعة المعالجة بـ D-gal عند المقارنة مع التحكم ، ALA و (D-gal + ALA) ، كما لم تكن هناك تغييرات كبيرة (D-gal (P≥0.05 بين مجموعات التحكم و ALA و (D-gal + ALA).

أظهر اختبار المتاهة المرتفع / الذراع المفتوحة زيادة معنوية (P≤0.05) بعد 30 يومًا في المجموعة المعالجة D-gal عند مقارنتها بالتحكم ، ALA و (D-gal + ALA) ، كما لم يكن هناك أي معنوية (P ≥0.05) تغييرات D-gal بين مجموعات التحكم و ALA و (D-gal + ALA). أظهر اختبار المتاهة المرتفع \ الذراع المغلقة انخفاضًا معنويًا (P≤0.0001) في المجموعة المعالجة D-gal عند مقارنتها بالتحكم ، ALA و (D-gal + ALA) ، أيضًا لم يكن هناك معنوي ((P≥0.0001 D- تتغير gal بين مجموعات التحكم و ALA و (D-gal + ALA).

في الختام ، أوضحت نتائجنا أن الدور المحسن لـ ALA في إجهاد الثور وتدهور السلوك والذاكرة في ذكور الجرذان كان ناتجًا عن D-gal.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء / كلية الطب البيطري

فرع الفسلجة والكيمياء الحياتية والادوية

دور حمض ألفا ليبويك في بعض المعاير الكيميو حيويه والسلوكية في الضرر العصبي المستحدث بواسطة د- جالاكتوز في ذكور الجرذان

رسالة مقدمة إلى

مجلس كلية الطب البيطري جامعة كربلاء في استيفاء جزء من متطلبات درجة ماجستير في الطب البيطري / فرع الفسلجة

بواسطة

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