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Effects of Atorvastatin and Streptozocin on Immunohistochemical Markers in Hippocampus of Male Adult Rats

A thesis

Submitted to the Department of Pharmacology and Toxicology and the Committee of Graduate Studies of the College of Pharmacy/ The University of Mustansiriya in Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacy (Pharmacology and Toxicology)

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We, the examining committee after reading this thesis, (**Effects of Atorvastatin and Streptozocin on Immunohistochemical Markers in Hippocampus of Male Adult Rats**), and examining the student **(Ala'a Adel Azeez Al-Hindawi)**, in its contents, find it adequate as a partial fulfillment of the requirements for the degree of M.Sc. in Pharmacy (Pharmacology and Toxicology).

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بسم الله الرحمن الرحيم

صدق الله العظيم

سورة طه (١١٤)

Dedication

To….

My mother for her support and scarified throughout my life "Words cannot express my gratitude toward you. I love you beyond words". All the love for my father too, thank you.

To….

My husband "my soul of my whole being", Ali, for his patient, support and help me in this study.

To….

My brother, Zaidoon, for his assist, help and support.

To….

My aunt, Dr.Luma, for her valuable advice and encouragement.

To….

My little angel, Rama, for inspiration and lighting of my life.

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Ala'a Adel Al-hindawi

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List of Abbreviations

4-AP	4-aminophenazone
25-OH	25-Hydroxycholesterol
7-K	7-Ketocholesterol
AchE	Acetylcholine Esterase
AchRs	Acetylcholine Receptors
AD	Alzheimer's Disease
ADP	adenosine-5-diphosphate
Akt	Protein Kinase B
ApoEε4	Apolipoprotein E Type 4
APP	Amyloid Precursor Protein
ATP	Adenosine Tri Phosphate
Aβ	Amyloid Beta
BACE	Beta-Amyloid Cleaving Enzyme
BBB	Blood Brain Barrier
CA	Cornuammonis
cGMP	Cyclic guanosine monophosphate
ChAT	Choline Acetyltransferase
CHE	cholesterol esterase
ChEIs	Cholinesterase Inhibitors
CHOD	cholesterol oxidase
CNS	Central Nervous System
Cox-2	Cyclooxygenase 2
CSF	Cerebrospinal Fluid
CYP	Cytochrome P
DAB	3-3'diaminobenzidine
DAP	dihydroxyacetone phosphate
DG	Dentate Gyrus
DNA	Deoxyribonucleic acid
eNOS	Endothelial Nitric Oxide Synthase
FAD	Familial Alzheimer's Disease
FPP	Farnesyl Pyrophosphate
GABA	Gamma-Aminobutyric Acid
GC	Guanylate Cyclase
GFAP	Glial Fibrillary Acidic Protein
GGPP	GeranylGeranyl Pyrophosphate
GluT2	Glucose Transporter 2
GPx	Glutathione Peroxidase
GPO	glycerol phosphate dehydrogenase
GR	Glutathione Reductase

GSH	Glutathione
GSK-3β	Glycogen Synthase Kinase 3β
GSSG	Glutathione Disulfide
G3P	glycerol-3-phosphate
H_2O_2	hydrogen peroxide
HDL	High Density Lipoprotein
HMG-coA	3-Hydroxy-3-Methyl Glutaryl Co-Enzyme A
HRP	Horseradish Peroxidase
ICV	Intracerebroventricular
IGF	Insulin like Growth Factor
IHC	Immunohistochemistry
IL	Interleukin
iNOS	Inducible Nitric Oxide Synthase
IRs	Insulin Receptors
LDL	Low Density Lipoprotein
LDL-c	Low Density Lipoprotein -cholesterol
LPL	lipoprotein lipase
LTP	Long Term Potetiation
MAP	Microtubule Associated Protein
MCI	Mild Cognitive Impairment
mtDNA	Mitochondrial Deoxyribonucleic Acid
NADPH	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
NF-Kappa-B	Nuclear Factor Kappa-B
NFTs	Neurofibrillary Tangles
NMDA	N-Methyl-D-Aspartate
nNOS	Neuronal Nitric Oxide Synthase
NO	Nitric Oxide
PBS	Phosphate Buffered Saline
PHF	Paired Helical Filaments
PI3K	Phosphatidylinositol-3-Kinase
PKC	Protein Kinase C
POD	peroxidase
PS	Presenilin Protein
RNA	Ribonucleic acid
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SD	Standard deviation
SES	Socioeconomic Status
SGZ	Subgranular Zone
SOD_3	Superoxide Dismutase
SORL1	Sortilin Related Receptor 1
STZ	Streptozocin

TG	Triglyceride
Tg	Transgenic
TNF	Tumor Necrosis Factor
VLDL	Very Low Density Lipoprotein
α7- nAChR	α 7- neuronal Acetylcholine Nicotinic Receptor
µl	Microliter
µm	Micrometer

Abstract

Background: Statins, beyond their lipid lowering role, exert beneficial effect by acting as a neuroprotective agent in some clinical cases such as brain injury, stroke, ischemia, seizures and Alzheimer's disease.

Objective: This study was designed to:

1- Investigate the effect of different oral doses of atorvastatin on normal male adult rats via studying immunohistochemical markers in the hippocampus.

2- Investigate the effect of different oral doses of atorvastatin on male adult rats model of Alzheimer's disease induced by 3 mg/kg streptozocin intrathecally via studying immunohistochemical markers in the hippocampus.

Materials and Methods: Forty eight adult male Wistar rats (200–250 gm) were used in the experiment. Animals were divided into 8 groups randomly each group contains 6 animals as follow: Group 1: Animals were administered saline orally for 30 days and serve as control group. Group 2: Animals were administered intrathecal injection of 3 mg/kg Streptozocin as a single dose and saline orally for 30 days. Group 3: Animals were administered intrathecal injection of 3 mg/kg Streptozocin as a single dose. At the same day, 5 mg/kg/day Atorvastatin were administered for 30 days. Similarly, rats in Group 4 and 5 were administered as in group 3 but with 10 mg/kg and 20 mg/kg atorvastatin respectively. Group 6: Animals were administered 5 mg/kg/day Atorvastatin for 30 days. Group 7 and 8 were administered atorvastatin orally for 30 days in doses of 10 mg/kg and 20 mg/kg respectively. All animals in this study were dissected, under anesthesia, at day 31.

Results: The results of this study showed a significant increase in the expression of glial fibrillary acidic protein in the hippocampus of rats

administered intrathecal injection of streptozocin when compared with control group. While, a significant reduction in that marker observed in the groups treated with a 20 mg /kg/day atorvastatin alone when compared with control and group administered 5 mg/kg atorvastatin. A significant reduction in this marker observed in the group treated with 20 mg/kg atorvastatin combined with a single intrathecal injection of streptozocin when compared with group administered streptozocin alone. Anti-oxidant state represented by glutathione reductase showed a significant increase in the expression of glutathione reductase in the hippocampus of rats treated with 20 mg/kg atorvastarin alone when compared with control one. A significant increase in the expression of this marker in the groups that administered 5, 10 and 20 mg/kg atorvastatin after intrathecal injection of streptozocin in compared with group administered streptozocin alone. However, a significant reduction in this marker observed in group administered streptozocin alone when compared with control group. A significant increase in the expression of neuronal nitric oxide synthase in the hippocampus of rats administered intrathecal injection of streptozocin compared with control group. Meanwhile, a significant reduction in that marker observed in the group treated with a 20 mg /kg atorvastatin alone when confronted with control group. Also, a significant reduction in this marker observed in the groups treated with 10 and 20 mg/kg atorvastatin in combined with a single intrathecal injection of streptozocin when compared with group administered streptozocin alone.

The results of this study shows that there are a significant improvements in the serum total cholesterol, LDL and TG as the dose of atorvastatin increased from 5 to 10 and to 20 mg/kg, while, atorvastatin does not change serum HDL level after 30 days of treatment. Furthermore, streptozocin injection has no effect on lipid profile.

Conclusion:

1. Administration of streptozocin intrathecally may yield a model of Alzheimer's disease indicated by brain damage which in turn improved by atorvastatin treatment.
2. Present study demonstrates that atorvastatin exerts its neuroprotective effects in a dose dependent manner.
3. Atorvastatin could be used for improving lipid profile; While, intrathecal injection of streptozocin has no effect on lipid profile.

Chapter One

Introduction

1.1. Hippocampus:

The hippocampus is the part of the brain which is located beneath the cortex within the inner folds of the medial temporal lobes (fig. 1-1). The hippocampus runs like a thick rope from one side of the brain to another. The two interlocking parts that make up this part of the brain are called the Ammon's horn and dentate gyrus. The hippocampus' appearance has been compared to a seahorse; The Latin term for the creature gives the hippocampus its name [1].

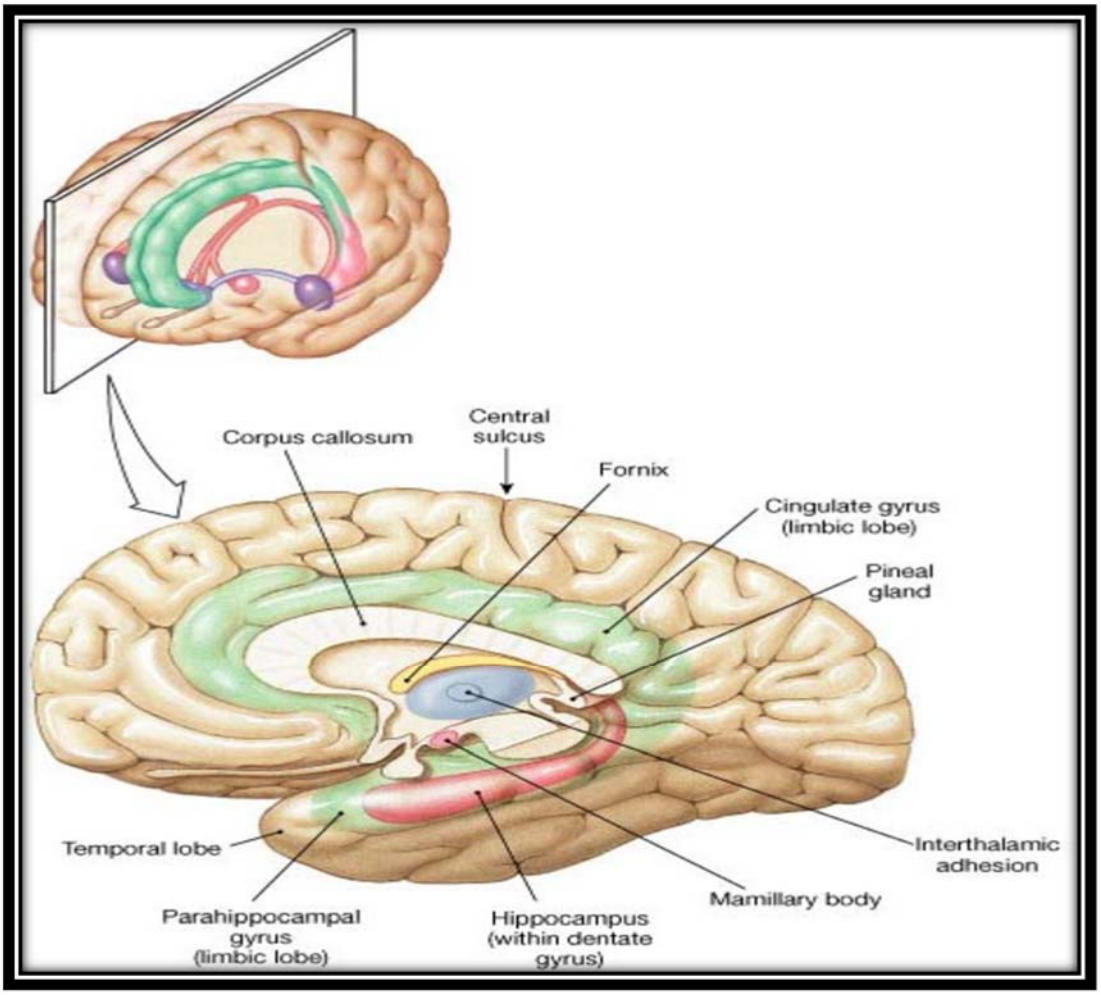

Figure (1-1): Limbic system, location of hippocampus (red), medial view [2].

1.1.1. Anatomy of hippocampus:

The mammalian hippocampus is comprised of four main regions: the Dentate Gyrus (DG), the Cornuammonis (CA), the Presubiculum and the Subiculum. The CA regions are further subdivided into four regions called CA1, CA2, CA3, and CA4 [(3)].

Together, the DG and CA regions make up the tripsynaptic hippocampal network (fig. 1-2). The first synapase is formed by excitatory input from the entorhinal cortex onto the dendrites of granule cell neurons of the DG. The entorhinal cortex is important for memory and navigation as the main interface between the hippocampus and neocortex [(3)]. In turn, these granule cells extend their axons from the DG to form the mossy fiber tract, which contacts the pyramidal neurons of the CA3 region. This connection represents the second synapse of the trisynaptic network. Pyramidal neurons of the CA3 region synapse to the pyramidal neurons of CA1 via the Shaffer collateral pathway to form the third synapse. Closing the network, pyramidal neurons of the CA1 region send axons to the subiculum which, in turn, project back to the entorhinal cortex. Importantly, adult hippocampal neurogenesis only generates new granule cells in the DG, which means it only has direct influences on the mossy fiber projections between the DG and the CA3 region [(4)].

Simultaneously, the trisynaptic hippocampal network offers a simplified view of the connections of the hippocampus. It is important to note that the Subgranular Zone (SGZ) and DG receive inputs from many different neurotransmitter systems which may influence adult hippocampal neurogenesis. Such inputs include glutamate from the entorhinal cortex, contralateral hippocampus, as well as from hillar mossy cells [(4)]. Other neurotransmitters include acetylcholine from the basal forebrain and septum, noradrenaline from the locus cereleus, GABA from local interneurons and

dopamine from the ventral tegmental area. Indeed, dopamine is effective in modulating the activities of newly generated hyperexcitable young neurons in the DG that are important for filtering incoming information [5].

The α7-neuronal acetylcholine nicotinic receptor (α7- nAChR) subtype is highly expressed at multiple loci and at different cell types in the hippocampus [6]. Compared with mature granule cells, newly generated neurons also have several unique characteristics that may indicate a specialized role in hippocampal function. The firing patterns of granule neurons of the DG are critical for encoding experience [7].

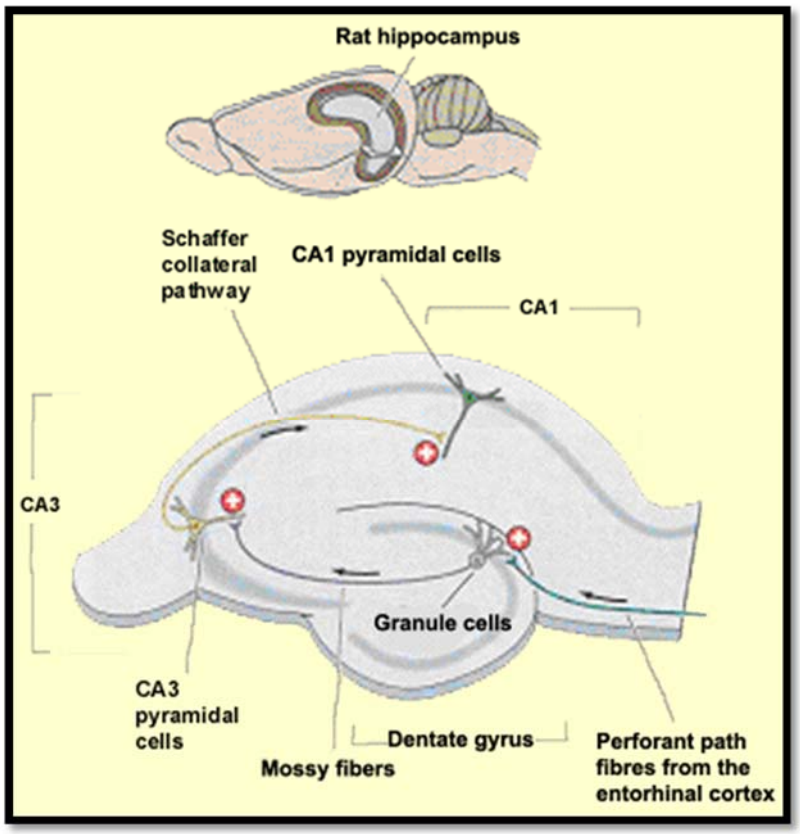

Figure (1-2): The trisynaptic loop of hippocampus [8].

1.1.2. Function of hippocampus:

The hippocampus has an important role in the formation of new memories about experienced events. Also, it plays a role in spatial memory and navigation. It is one of the structures within the brain that makes up the limbic system, which is responsible for emotions, memories, motivation and other "preconscious" functions [(9)].

Historically, hippocampus function was thought to be responsible for the sense of smell, but that theory has since been disproven. It is now known to control the memory of smell, and not the sense of smell itself. Without a fully functional hippocampus, humans may not remember where they have been and how to get where they are going [getting lost is one of the most common symptoms of amnesia] [(10)].

1.1.3. Hippocampus changes in Alzheimer disease:

Hippocampus is one of the first regions affected by changes in the brain of Alzheimer's disease (AD) patients. Alzheimer's disease causes two distinct deformities in the hippocampus, neurofibrillary tangles and senile plaques [(11)].

The neurofibrillary tangles are found in the cytoplasm of neurons in the entorhinal cortex. There are two different kinds of plaques, neuritic and diffuse [(12)]. Neuritic plaques are spherical structures that contain neurites, which are surrounded by an abnormal protein known as amyloid. Diffuse plaques lack neurites and have an amorphous appearance [(13)].

As the number of plaques and tangles increases, healthy neurons begin to function less effectively [(14)]. Gradually, the neurons lose their ability to communicate and consequently die, resulting in an overall shrinkage of brain

tissue, particularly in the hippocampus, that lead to restrict the patient's ability to form new memories[15].

1.2. Alzheimer's disease:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder representing the most common cause of dementia in the elderly population. This disease was documented more than one hundred years ago by the German psychiatrist Dr. Alois Alzheimer [16].

1.2.1. Epidemiology:

In 2010, 35.6 million people worldwide suffered from dementia with AD, accounting for 60 to 80 percent of the cases. Due to the international increase in life expectancies, the number of AD patients is predicted to increase dramatically in the near future and by 2050, one new case of AD is expected to develop every 33 seconds, or nearly a million new cases per year [17]. Between 2000 and 2010 the proportion of deaths resulting from heart disease, stroke, and prostate cancer decreased to 16%, 23%, and 8%, respectively, whereas the proportion resulting from AD increased to 68%[17].

1.2.2. Alzheimer's Risk Factors:

Age is the most obvious risk factor for AD. The prevalence of AD increases with age from 4% in the 65 to 75 years age group to 19% in the 85 to 89 years age group, and the incidence of AD increases from 7/1000 in the 65 to 69 years age group up to 118/1000 in the 85 to 89 years age group [18]. Studies indicate that people age 65 and older survive an average of four to eight years after a diagnosis of Alzheimer's disease [19]. Family history is another potent AD risk factor. In terms of genetic influences, AD is a

heterogeneous disease that can be classified as either familial or sporadic. Familial AD is autosomal dominant and typically presents before age of 65 with symptoms becoming evident as early age of 28 [20]. Cases of familial AD are rare, with prevalence below 0.1% [21]. Most cases of familial disease result from mutations in the amyloid precursor protein (APP), presenilin 1 gene (chromosome 14) and presenilin 2 gene (chromosome 1)[22]. In terms of sporadic AD, the apolipoprotein E (ApoE ε4) allele accounts for most of the genetic risk [23]. Besides age and genetics, the next most important AD risk factor is level of attained education. Numerous studies have reported an increased risk of AD among participants with lower levels of formal education [24]. It is speculated that education may act, along with other ―life course influential factors [25], such as occupation and early-life household socioeconomic status (SES), to modify other AD risk factors (e.g., brain size) and subsequent clinical manifestation [26,27].

Several other potential AD risk factors exist that fall into the category of lifestyle factors. These risk factors may influence AD development by means of vascular mechanisms, and include hypertension, diabetes mellitus, and hyperlipidemia [28]. Several studies have related hypertension to brain atrophy, white matter lesions, and neurofibrillary tangles, while in term of DM, A higher circulating blood glucose level is toxic to nerve cells, as it causes protein glycation and oxidative stress [29]. Smoking has also been identified to be a risk factor for AD. A recent meta-analysis examining 14 non-tobacco industry-affiliated cohort studies revealed smokers to have a significantly increased AD risk compared with non-smokers [30]. In addition, smoking is hypothesized to contribute to AD neuropathology through oxidative stress. Since smoking is related to several vascular factors, it can also be conceptualized as a vascular risk factor for AD [31]. Other vascular factors, such as hypercholesterolemia and hypertension, have also been

associated with AD [32, 33]. It is hypothesized that these risk factors may contribute to cerebral hypo-perfusion, resulting in clinical AD symptoms [34].

1.2.3. Etiology of Alzheimer's disease:

Alzheimer's disease pathology is characterized by the formation and accumulation of misfolded proteins (plaques and tangles) in the brain.

1.2.3.1. Formation and accumulation of misfolded proteins:

Amyloid β

The formation of extracellular plaques is described by the amyloid cascade (also Aβ-protein) theory of plaque causing pathological changes [35]. Plaques, which are extracellularly formed, those arise when the amyloid precursor protein (β-APP) is cleaved by the beta-amyloid cleaving enzyme (BACE) (especially by β- and γ-secretases) to form the predominant peptides, Aβ40 and Aβ42. Although, the levels of Aβ40 peptides are significantly higher than those of Aβ42, the latter form is more toxic, which has a much higher propensity to aggregate, and is believed to be crucial in initiating amyloid formation and the pathogenesis of Alzheimer's disease [36].The presenilin proteins (PS1 and PS2) are critical in the enzymatic cleavage of the APP, and subsequent release of β-APP. Specific mutations in the presenilin genes may result in familial Alzheimer's disease (FAD), through an increase in APP cleavage, which causes an increase of β- amyloid [37]. FAD research has shown that the allele apolipoprotein E type 4 (APOE ε4) increases the risk of late onset Alzheimer's disease. Several studies have suggested contemporary environmental conditions which may have led to APOE ε4 carriers to have an increased susceptibility in developing AD, such as high intake of carbohydrates and fat, low fiber, and reduced physical activity [38].

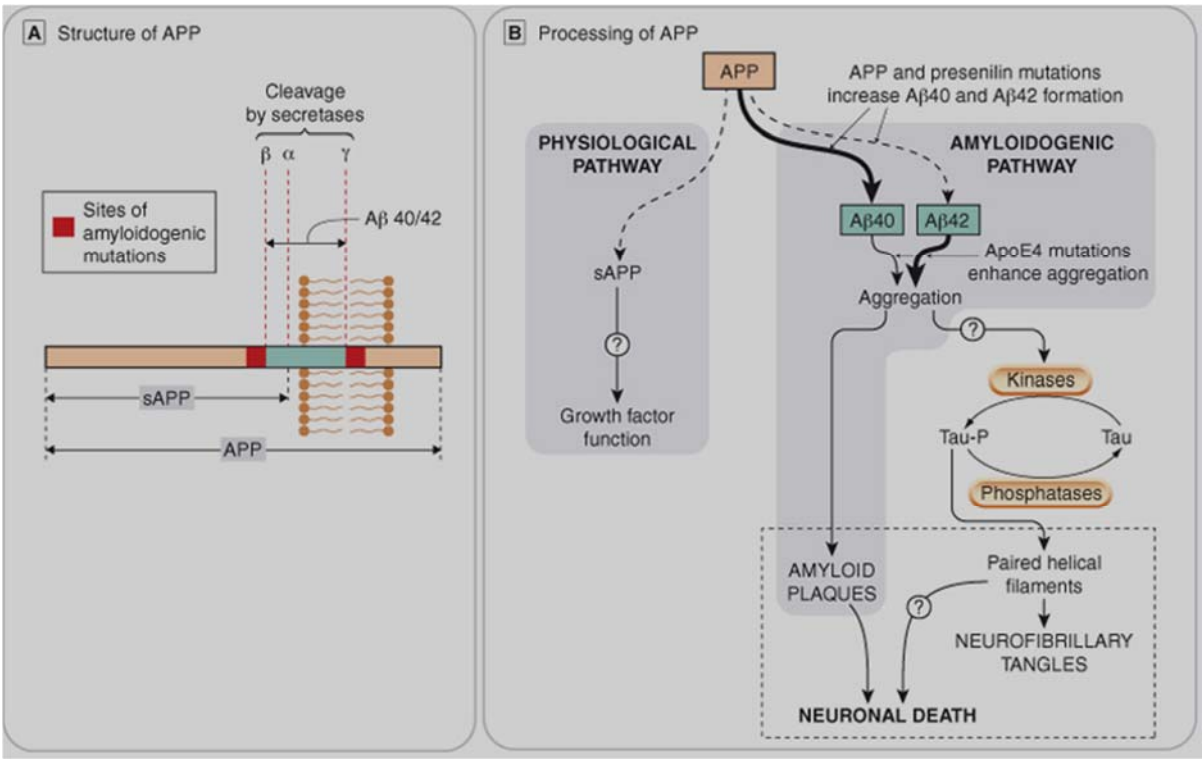

Figure (1-3): The amyloid cascade hypothesis [39].

The amyloid cascade theory is thought to be relevant to genetically inherited or predisposed people, where by a ready mutation must exist [35]. However, the theory does not explain the cause of sporadic Alzheimer's disease. Plaques are also common in non-demented individuals [35].The sortilin related receptor 1 (SORL1), is a neuronal sorting receptor which controls APP processing. SORL1 works by directing APP into the recycling pathway, and thus away from enzymatic cleaving by BACE and the presenilin proteins. As BACE and the presenilin proteins cannot act on APP, this results in a reduction of Aβ production. However, where an under expression of SORL1 occurs, then a direct transit of APP to BACE occurs, which results in Aβ production. Because there is no known reduction in SORL1 in FAD, SORL1 may be the first gene that is linked to sporadic Alzheimer's disease [40]. Levels of SORL1 have been shown to be reduced in the brains of Alzheimer's disease

patients [40] and in the brains of individuals with mild cognitive impairment [41].

Clusterin is a chaperone protein involved in the production of Aβ. A recent genome wide association study in patients with Alzheimer's disease found that clusterin was associated with the severity and progression of Alzheimer's disease [42].

In the progression of AD, it has been demonstrated that accumulation and aggregation of Aβ peptide in the hippocampus of the brain usually results in the activation of glial cells [43] which, in turn, initiates a neuroinflammatory response, involving reactive oxygen intermediates and inflammatory cytokines, including interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF-α) [20,44] .

Aβ peptides, together with altered mitochondrial function, and the presence of trace metal ions such as iron and copper, have been identified as potential sources of oxidative stress [45, 46]. Consistent with the Aβ-induced oxidative stress hypothesis, oxidative stress is the result of Aβ insertion as oligomers into the bilayer causing reactive oxygen species (ROS) production and initiating lipid peroxidation and protein oxidation in AD pathogenesis [47,48].

Tau

The second major hallmark of Alzheimer's disease related changes in the brain are intracellular formations called neurofibrillary tangles (NFTs). Neurofibrillary tangles are primarily composed of paired helical filaments (PHF). The major component of the NFTs is the protein tau, a microtubule associated protein (MAP), and this binds with microtubulin to provide structural stability to a cell. Dissociation of the tau protein from the microtublin leads to unbounded tau protein aggregation. The reason for the aggregation is explained by the tau hypothesis [49]. Under normal conditions,

tau is a soluble protein that undergoes phosphorylation and dephosphorylation, thus forming insoluble aggregates. An imbalance in this dynamic results in increasing levels of abnormally hyperphoshorylated tau (P-tau 181, P-tau 199, P-tau 231, P-tau 396, P-tau 404), which in turn sequesters normal tau and other MAPs (MAP1 and MAP2) [50]. Hyperphosphorylated tau aggregates into PHF, and tangle formation.

Parallel to the process of tangle formation is the disassembly of microtubules. The combined effect of tangle formation and disassembly of microtubules is that they compromise normal neuronal and synaptic function. According to the amyloid cascade hypothesis, it is the increase in the concentration levels of Aβ that trigger the changes in tau thus leading to the formation of NFTs[51].

As microtubule function is necessary for normal neuronal and synaptic function, dysfunction of the microtubules may be central in neurodegeneration. The number of neurofibrillary tangles is a pathological marker of Alzheimer's disease severity [52].

1.2.3.2. Oxidative Stress:

Several studies suggest that mitochondrial oxidative damage is an early event and plays a key role in the progression, pathogenesis, and consider to be a hallmark of neurodegenerative diseases [53, 54]. Recent AD postmortem brains, transgenic mouse models and cell models of AD revealed that Aβ significantly induces DNA damage in age-dependent manner in neurons from patients with AD or AD-like animal models [55]. Aβ also induces oxidative mtDNA damage, in turn, generates excessive free radicals, and causes more DNA damage, it likely forms a vicious cycle [55].

Indeed, there are strong indications that oxidative stress occurs prior to the onset of symptoms in AD and oxidative damage is found not only in the

vulnerable regions of the brain that is affected by the disease [56] but also peripherally [57]. The central nervous system (CNS) is particularly susceptible to reactive species-induced damage for several reasons: First, it has a high consumption of oxygen. Seconds, it contains high levels of membrane polyunsaturated fatty acids susceptible to free radical attack and finally, it is relatively deficient in oxidative defenses [58].

1.2.3.3. Inflammation:

Brain regions which are affected by Alzheimer's disease are known to contain increased neuroinflammatory mediators (cytokines and microglia) through increased inflammatory cascades [59]. Whether this is a natural response to control inflammation or an out of control to immune process, is unknown. In the Alzheimer's disease brain, amyloid deposition provokes the phenotypic activation of microglia (which is the brain's tissue macrophage and representative of the innate immune system. These cells normally provide tissue maintenance and immune surveillance of the brain) and their elaboration of proinflammatory molecules [60]. Recent evidence suggests that inflammatory mechanisms represent a third component (besides amyloid beta peptides and neurofibrillary tangle formation) and once initiated by degeneration, may significantly contribute to disease progression and chronicity [61]. Various neuroinflammatory mediators including complement activators and inhibitors, chemokines, cytokines and inflammatory enzymes are generated and released by microglia, astrocytes and neurons. While release of these factors is typically intended to prevent further damage to CNS tissue, they may also be toxic to neurons and other glial cells [62].

1.2.3.4. Cholinergic hypothesis:

The cholinergic hypothesis of Alzheimer's disease suggests that destruction of the cholinergic pathway in the basal forebrain results in a reduction of cholinergic neurons, which release the neurotransmitter acetylcholine. These neurons project to the hippocampus and neocortex, which are implicated in both memory disturbance and cognitive symptoms [(63)].The dysfunction of the cholinergic system in AD occurs at various levels including a decreased choline acetyltransferase activity, reduced choline uptake, a decreased in acetylcholine synthesis and altered levels of acetylcholine receptors (AChRs)[(64)]. In individuals having Alzheimer, the activity of the acetylcholinesterase increases that leads to increase breakdown of the neurotransmitter acetylcholine which in turn cause a reduction in the acetylcholine level in the brain. Another relation between acetylcholinesterase and AD has been the partial involvement of the enzyme in the formation of amyloid plaques and neurofibrillary tangles. It has been shown that AChE formed a complex with the growing fibrils that lead to induce the aggregation of β-amyloid peptide fragments. These complexes have been shown to be more cytotoxic than β-amyloid fibrils alone [(65)].

Alpha-7 neuronal nicotinic acetylcholine receptors (α7 nAChRs) are homopentamer, ligand gated cationic channels. They are widely expressed in the central nervous system with high levels in the regions relevant to memory functions and involved in processing of sensory information, such as hippocampus [(66)]. It has been demonstrated that Aβ binds to α7 nAChR with high affinity and they both are present in senile plaques [(67)]. Their interaction alters several neurochemical processes including Ca^{2+} homeostasis and acetylcholine release. Thereby modulates neuronal physiological functions

implicated in memory processes. Chronic inhibition of cholinergic signaling by Aβ could be attributed to the cognitive deficits associated with AD [68].

1.2.3.5. Cholesterol metabolism:

The brain comprises 25% of the body's total cholesterol pool. However 2% of the body's total weight and all the cholesterol that is found in the brain is synthesized there [69]. Brain cholesterol is at a concentration of 15-20 g/kg of tissue weight, which is mostly unesterified and predominantly concentrated within the myelin that serves to insulate neuronal axons and thus increase the efficiency of their electrical signaling. Unlike in other tissues, brain cholesterol has a slow turnover rate, with a flux rate of only 0.9% of that normally seen across the entire body, which translates into a half-life of 4-6 months in the rat brain and 6-12 months in a human [70, 71, 72].

Free cholesterol in the brain, if allowed to accumulate, is toxic to cells because of its amphipathic nature [69]. Unlike other tissues, the mechanism by which cholesterol is removed in the brain differs somewhat because of the presence of the blood brain barrier (BBB) that means Cholesterol can undergo oxidative modifications at least by two mechanisms: a direct radical attack involving reactive oxygen species ROS or reactive nitrogen species RNS (non-enzymatic mechanism), or by the activity of a specific enzymes (enzymatic mechanism) [72]. Cholesterol oxidation leads to the formation of oxysterols. The latter moieties are major regulators of cholesterol homeostasis in the central nervous system [72]. Among oxysterols, 7-ketocholesterol (7-K) and 25- hydroxycholesterol (25-OH) have shown to cause apoptotic neuronal death by inducing mitochondrial dysfunction [73]. Cholesterol can also be removed to a much lesser extent by apolipoproteins that are transported to the CSF [72].

The role of lipid/cholesterol metabolism and Alzheimer's disease pathogenesis is gaining acceptability [29]. Cholesterol is known to affect the activity of enzymes involved in the metabolism of APP in the production of Aβ[74]. Apolipoprotein (APOE) is involved in the transporting of cholesterol, and APOE ε4 allele is a universally accepted marker which increases Alzheimer's disease risk [75]. APOE ε4 is also associated with lowering the age of onset for Alzheimer's disease [29]. APOE ε4 does not only confer a genetic risk for Alzheimer's disease to an individual, but it is also linked to the production and aggregation of both amyloid and tau [76].

Specifically the ε4 allele is associated with increased amyloid burden and dysfunction of cholinergic neurotransmission. Individuals who inherit two ε4 alleles (homozygotes) have higher risk of developing Alzheimer's disease than heterozygous carriers [38]. A high cholesterol level during an individual's mid-life is considered a risk factor for Alzheimer's disease [77].

1.2.4. Clinical features:

It is believed that the neurodegenerative processes of AD are already ongoing for 20 to 30 years before the appearance of clinical symptoms, which termed the "preclinical stage" [78, 79]. At some point in time, sufficient brain damage accumulates to result in cognitive symptoms and impairment. This has been called mild cognitive impairment (MCI), a condition in which subjects are usually only mildly impaired in memory with relative preservation of other cognitive domains and functional activities and do not meet the criteria for dementia [80], or as the prodromal state AD [78].Several studies showed that MCI patients progressed to AD at a yearly rate of 10% to 15%, and that predictors of this conversion included whether the patient was a carrier of the ε4 allele of the apolipoprotein E (APOE) gene, clinical severity, brain atrophy, certain patterns of CSF biomarkers of cerebral glucose metabolism, and Aβ

deposition [80]. MCI patients often display subtle problems with attention and executive function, abstract thinking and impairments in semantic memory, even though their daily living is not affected by it [81].

With progression of the disease, other cognitive domains are affected, leading to more severe symptoms including confusion, irritability, aggression and mood swings. The progressive impairment of learning and memory in the patients requires a close supervision of their daily lives as they are unable to plan, judge, and organize tasks [82]. At the late disease stage, the memory is impaired to a degree that even biographical memories cannot be recalled. Also, language is severely impaired, with patients using only simple sentences or even just words to articulate their needs [82]. Occurrence of motor impairments is also commonly observed in AD patients, ranging from poor facial expression, rigidity and posture/gait to bradykinesia [83].

1.2.5. Pharmacotherapy:

Current treatments for Alzheimer's disease are used to reduce the cognitive decline. The central role of these drugs is to stabilize and thus minimize disruption of two key neurotransmitters, acetylcholine (ACh) (the cholinergic hypothesis of Alzheimer's disease), and glutamate. AChE inhibition is used to protect the cholinergic neurons [84].The three compounds which work on the basis of AChE inhibition are the cholinergic drugs, donepzil, rivastigmine and galantamine. All three compounds are efficacious in reversing and improving memory and global cognition, in mild to moderately demented patients [85]. The second key transmitter targeted is glutamate, the primary excitatory neurotransmitter in the brain. The interaction of glutamate with the *N*-methyl-D-aspartate (NMDA) receptor is important in working of memory and learning. In Alzheimer's disease an increase in glutamate activity results in NMDA receptor being excessively activated which may lead to neurodegeneration [84]. Consequently, memantine an

NMDA antagonist is used to counter the loss or damage of NMDA receptors due to excess glutamate excitation in Alzheimer's disease patients. Targeted primarily at moderate to severely demented patients, memantine is considered to overall reduce burden of care on the career, as well as clinically reversing and improving memory and global cognition, reducing behavioral disturbances, and improvement in the quality of life [86]. Recently, many drugs have been claimed to improve cognitive performance and several new approaches are being explored; Inhibitors of β- and γ-secretase have been identified and are undergoing clinical trials. Furthermore, work on developing immunization strategies is continuing to prevent and reverse plaque formation. Epidemiological studies reveal that some non-steroidal anti-inflammatory drugs might reduce the likelihood of developing AD, Ibuprofen and indometacin have this effect. Moreover, the amoebicidal drug "clioquinol" is a metal-chelating agent that causes regression of amyloid deposits in animal models of AD, and showed some benefit in initial clinical trials. There are other approaches under investigation, such as implanting cells engineered to secrete nerve growth factor in the brain [39].

1.3. Role of statins in Alzheimer's disease

1.3.1. Background:

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are a well-established class of drugs used in the treatment of hypercholesterolemia. High plasma LDL-C is a risk factor of cardiovascular diseases [18,87]. Statins are recommended as first-line therapy for hypercholesterolaemia [88], since they have shown to reduce the risk of cardiovascular morbidity and mortality in patient with or at risk of coronary heart disease in several clinical trails [89,90].

Statins may be classified into three categories based on their increasing potency and efficacy in reducing low density lipoprotein cholesterol (LDL-C). First generation statins include lovastatin, pravastatin, and fluvastatin. Second generation statins include, simvistatin and atorvastatin. Third generation statins include, rosuvastatin [91]. Atorvastatin, which belongs to the second generation of statins (fig. 1-4), is a synthetic reversible inhibitor of HMG-CoA reductase. The dosage range used clinically is 10-80 mg/day [92].

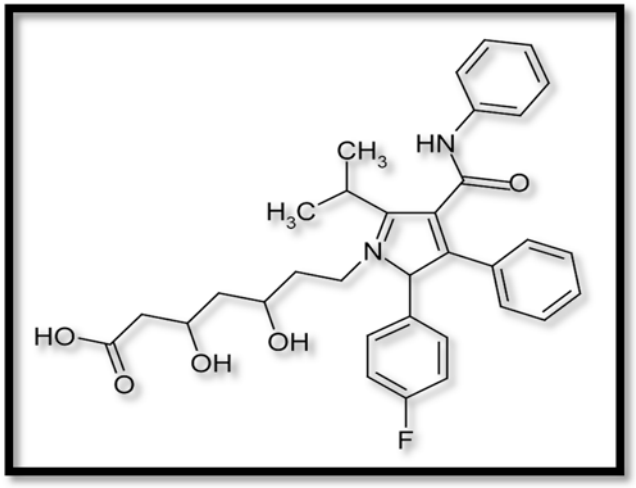

Figure (1-4): Chemical structure of atorvastatin [87].

1.3.2. Mechanism of action:

Statins act by inhibiting of HMG-CoA reductase enzyme leading to block the conversion of HMG-CoA to mevalonate, a rate-limiting step in cholesterol biosynthesis [93, 94] as shown in figure (1-5). Low-density lipoprotein (LDL) - cholesterol synthesis decreases in hepatocytes, as a result of statin administration, and this reflects a reduced cholesterol blood level. In addition to this effect, statins have shown to increase HDL-cholesterol and reduce triglyceride plasma levels [94].

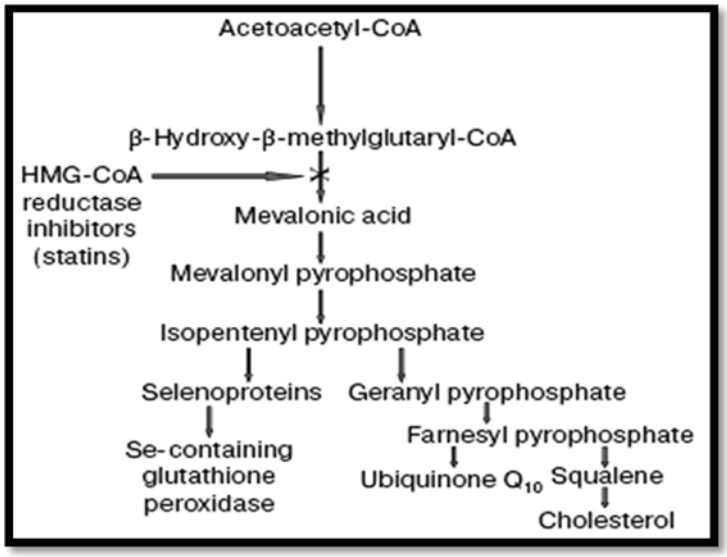

Figure (1-5): Statin's mechanism of action [95].

1.3.3. Statins' pharmacokinetics and pharmacodynamics profile:

Although, all statins share the same main mechanism of action, their pharmacokinetic profile is quite different. All statins are well absorbed by the intestine when given orally, even though they undergo marked first-pass effects in the liver, which reduces the systemic bioavailability (5–30%); upon administration, statins reach peak plasma concentration, ranging from 10 to 448 ng/ml, within 0.5–4 h. In the plasma, statins are bound to albumin (43–99%) and this binding account for their variable half-life. Atorvastatin and rosuvastatin are the statins with the longest half-life (15–30 and 20.8 h, respectively) [93]. Statins generally are metabolized by the liver through the isoforms 3A4 (atorvastatin,lovastatin and simvastatin) and 2C9 (fluvastatin and rosuvastatin) of the cytochrome-P-450 (CYP) system. The primary route of elimination is fecal, and only a minor fraction of statins is eliminated via urine [93, 94].

1.3.4. Tolerability and adverse effects:

In general, statins monotherapy is well tolerated with a low frequency of adverse events. The common adverse effects associated with statins therapy are relatively mild and often transient, such as gastrointestinal symptoms

(nausea and abdominal discomfort), headache and rash. The most important adverse effect associated with statins is myopathy which occurs infrequently [96].

The term myopathy designated any non-inherited disorder of skeletal muscle that causes proximal muscle weakness. In statin clinical trails, the reported incidence of myopathy is as low as 0.1 to 0.2% [97]. The symptoms may progress toward rhabdomyolysis as long as patients continue to take the drug. Rhabdomyolysis is a syndrome that results from severe skeletal muscle injury and lysis, leading to a marked increasing of Creatinine Kinase (in excess of 10 times the upper limit of normal) often accompanied by evidence of renal dysfunction and occasionally renal failure and death. [96]. The detailed mechanisms by which statins cause myopathy is not fully understood, but some hypothesis have suggested that inhibition of HMG-CoA reductase may directly cause this myotoxicity [98]. The clinical association appears to be dose dependent, and the risk is known to increase when statins are prescribed in combination with agents that increase the serum concentration of the statins [99], for this reason; statins should not be taken with inhibitors of CYP3A4 such as azole antifungals, erythromycin, ritonavir and grapefruit juice. Also the association statins and fibrates should be avoided, in particular gemfibrozil [94].

1.3.5. Atorvastatin as neuroprotective agent:

Although cholinesterase inhibitors (ChEIs) are still recommended as the primary drug of choice for AD and related diseases, their efficacy is frequently questioned. Recent studies reported that α7-neuronal acetylcholine nicotinic receptor (α7-nAChR), which is located on perivascular postganglionic sympathetic nerve terminals, mediated neurogenic vasodilatation of porcine cerebral arteries was blocked by ChEIs, and this

blockade was prevented by statin pretreatment leading to activation of this receptor that is eventually result in nitric oxide release and vasodilatation by acting on presynaptic β2-adrenoceptors located on neighboring nitrergic nerve terminals [100].

From a general point of view, the neuroprotective effects of statins include: (i) the inhibition of endothelial $O_2^{\cdot-}$ formation by preventing the isoprenylation of p21 Rac, which is critical for the assembly of NADPH oxidase after activation of PKC [101]; (ii) the increase of SOD_3 activity as well as the number of functionally active endothelial progenitor cells [102]; (iii) the increase of the expression of endothelial nitric oxide synthase (eNOS) by inhibition of Rho isoprenylation [103] and (iv) the activation of eNOS via post-translational mechanisms involving activation of the PI3K/Akt pathway [104].

Statins not only lower cholesterol but they also showed positive effects against Alzheimer-relevant amyloid beta-induced oxidative stress in mice models of AD [105,106] as well as a reduction in CSF tau protein phosphorylation in humans [107]. Statins reduced amyloid beta production by decreasing Aβ secretion, and inhibiting the protein isoprenylation and Rab/Rho membrane localization, which lead to the reduced levels of amyloid precursor protein (APP) C-terminal fragments due to enhanced lysosomal dependent degradation [108]. In a six-month AD anti-inflammatory prevention trial, profoundly reduced risk of incident AD with lower cholesterol level was observed to be associated with statin medication [109]. Atorvastatin has demonstrated its anti-apoptotic effects by blocking Aβ 1-42 induced neuronal death as well as reduced caspase-3 activity [110]. Besides lowering serum cholesterol and mediating the metabolisms of amyloid beta peptides, statins also influence the development of AD via interacting with atherosclerosis and apolipoprotein E (ApoE) [111].Two cross-sectional studies showed that

compared with non-ApoE phenotype carriers, cognitive decline and poorer memory performance experienced more pronounced deterioration in AD patients with ApoE isoforms, and atorvastatin induced a beneficial effect on them [112, 113]. In a population-based cohort study, statins were found to ameliorate the impaired cognition in older participants who had increased atherogenic lipoproteins [114].

Iiterleukin-1β, IL-6, and TNF-α were significantly decreased in the atorvastatin-treated AD people. And it might attenuate the damage of nerve cells; improve learning and memory ability, by inhibiting inflammatory response in the progression of AD [43]. Additionally, rats treated with atorvastatin *in vivo* for 3 weeks showed increased production of the anti-inflammatory cytokine interleukin-4 in the hippocampus, and they were protected against deficiency in long term potentiation(LTP) caused by acute injection of the amyloid-beta (Aβ) peptide, Aβ1–42 [115]. Recent studies shown that atorvastatin may act by reduction in the hippocampal astrogliosis, lipid peroxidation and COX-2 expression[116], as well as an increase in glial glutamate transporters expression [116, 117].

Horsdal H.T *et al*. (2009) found that statin users with dementia were less likely to be hospitalized than non statin users. Their data suggests that long term statin use might reduce morbidity in persons with dementia but there was no mention of a dose response [118]. Studies have also suggested that high levels of cholesterol perhaps contributing to pathology that closely resembles AD. Since statin use may reverse the effects of cholesterol, it may be possible to use statin treatments to prevent or treat AD [119].

1.3.6. Statins induce neuronal damage:

Some studies of effects of statins on the brain and brain cells suggested that statins have potential not only to promote health but also to induce harm in the central nervous system. For example, a recent study by Coetsee *et al*. (2008) had shown that statins induce significant DNA damage in neuronal cells *in vivo* [120]. Other studies reported activation of both pro- and anti-inflammatory pathways, increased cell death, and higher susceptibility to oxidative damage in the brain tissue or brain cells exposed to statins [121,122].

3-Hydroxy-3-methylglutaryl coenzyme A reductase converts HMG-CoA into mevalonate, and inhibition of HMG-CoA reductase, by statins, reduces the synthesis of all mevalonate pathway products. Indeed, mevalonate is a precursor of not only cholesterol, but also of many non-steroidal isoprenoids, including farnesyl pyrophosphate (FPP) and geranyl geranyl pyrophosphate (GGPP). Isoprenylation is a functionally important post-translational modification of a variety of proteins, including small GTPases (*e.g.* Ras, Rab, and Rho). It plays a crucial role in protein trafficking and signaling, cytoskeletal structure, cell motility, and membrane transport [123, 124]. The low isoprenoid levels cause cell-associated accumulation of APP fragments, and intracellular Aβ, also inhibit the trafficking of APP through the secretory pathway. Assuming that the rates of APP protein synthesis and degradation remain unchanged, reduced transport of APP through the secretory pathway would lead to elevated levels of APP in biosynthetic compartments (*i.e.* Endoplasmic Reticulum) [125].

Despite the fact that memory impairment is rarely reported as an adverse effect of statin therapy, the statin-induced memory problems are real, potentially causing a substantial amount of concern and confusion to the

patients. The effects of statins on the brain are far less well understood than their effects on other target organs [125].

1.4. Intracerebroventricular streptozocin induced Alzheimer's disease in animal's model:

Streptozocin (STZ), originally identified in the late 1950`s as an antibiotic, is a naturally occurring compound that is produced by the bacterium *Streptomyces achromogenes* and shows broad spectrum antibacterial properties [126].

Streptozocin is used to generate experimental diabetic animal models by selectively causing beta-cell destruction, since it is transported through GLUT2, which is expressed relatively high in beta cells [127, 128]. The multiple low-doses of STZ administration (30, 35, 40 mg/kg body weight) are one of the most widely used approaches for generating insulin-deficient-dependent diabetic animal models [129]. Streptozocin is used to treat metastatic cancer of the pancreatic islets. However, it is only used under the circumstances that the cancer cannot be removed by surgery [130]. However, STZ is not a drug of choice for treatment of cancers due to development of resistance to its effect on the genes and DNA of the beta cells. Moreover, severe toxicities were observed in different cancer patients when STZ was used alone or in combination with other antineoplastic drugs [130].

1.4.1. Central mechanism of action of streptozocin:

Central STZ (Fig.1-6) administration caused neither systemic metabolic changes nor diabetes mellitus, but developed numerous behavioral, neurochemical and structural features that resembled those found in human AD [131]. Streptozocin has been administrated mostly in doses ranging from 1–

3 mg/kg body weight, injected 1–3 times, either uni-or bi-laterally into the lateral cerebral ventricles. Identical biochemical changes have been found in the left and right striatum after administration of STZ into the right lateral cerebral ventricle only [132].

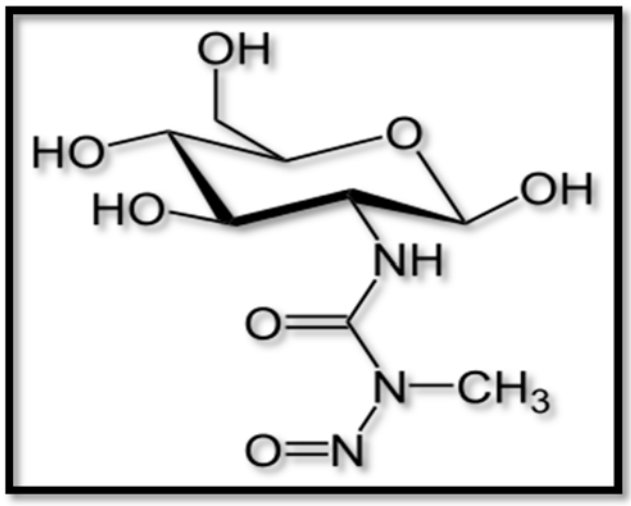

Figure (1-6): Chemical structure of streptozocin [133].

A- Intracerebroventricular streptozocin induced insulin signaling alteration:

Substantial evidence has been gathered in support of the presence of both insulin and insulin receptors in the brain. The main source of brain insulin is the pancreas, crossing the blood–brain barrier by a saturable transport mechanism [134].

The intracerebroventricular -STZ-injected rats did not have elevated blood glucose or insulin levels, and pancreatic architecture and insulin immunoreactivity were similar to control. Yet their brains were atrophied and had striking evidence of neurodegeneration with cell loss, gliosis, and increased immunoreactivity for p53, activated GSK-3β, phospho-tau, ubiquitin, and APP-Aβ[135]. Treatment with very low to moderate doses of STZ in short term experiments causes insulin resistance via a decrease in

autophosphorylation and in total number of IRs [136]. Also, reduced expression of genes encoding insulin, IRs, and insulin receptor-substrate 1 and reduced ligand binding to the insulin in CA3 region of hippocampus [137].

B- Intracerebroventricular streptozocin induced oxidative stress:

Streptozocin is 2-Deoxy-2{[methylnitrosoamino)carbonyl]amino} D-glucopyranose, i.e., a nitrosamidemethylnitrosourea linked to the C2 position of D-glucose (fig. 1-6). Once metabolized, the N-nitrosoureido is liberated and causes DNA damage through generation of reactive oxygen species such as superoxide, hydrogen peroxide, and nitric oxide in brains of ICV -STZ treated rats [138].

Potential sources of oxidative stress in AD and the ICV-STZ model include mitochondrial dysfunction [139] and impaired insulin/IGF signaling [140].

C- Intracerebroventricular streptozocin induced neurotransmission deficits:

Intracerebroventricular -STZ treated rats showed an impaired learning and memory performance, and leads to cognitive dysfunction by inhibiting the synthesis of adenosine triphosphate (ATP) and acetyl-CoA. This ultimately results in a cholinergic dysfunction supported by reduced of hippocampal choline acetyltransferase (ChAT) activity in rats one week after ICV -STZ injection and is still present 3 weeks post-injection[141]. This is followed by a significant increase in acetylcholinesterase (AChE) activity [140].

D- Intracerebroventricular streptozocin induced behavioral alterations and structural changes:

Intracerebroventricular-STZ treated rats consistently demonstrate deficits in learning, memory, and cognitive behavior that is observed as early

as 2 weeks after administration and reported to still persist 12 weeks post treatment [138,142,143].A direct histopathological evidence, caused by STZ by its specific neurotoxic damage to axon and myelin in some brain regions responsible for learning and spatial memory including the fornix, anterior hippocampus and peri-ventricular areas, have been reported [144].

E- Decreased glucose/energy metabolism:

Intracerebroventricular administration of STZ clearly shows heterogeneous changes in local cerebral glucose / energy utilization, particularly in cerebral cortical regions and hippocampus, it has been reported 3 weeks following the administration [145].

1.5. Immunohistochemistry theory:

Immunohistochemistry (IHC) is a technique for identifying cellular or tissue constituents (antigens) by means of antigen-antibody interactions. The site of antibody binding is being identified either by direct labeling of the antibody or by using a secondary labeling method; immunohistochemistry is a powerful technique that is widely used in clinical histopathological diagnosis and for assessing the progression and treatment of diseases such as cancer [146].

1.5.1. Glial Fibrillary Acidic Protein:

Glial fibrillary acidic protein (GFAP) is the principal 8-9 nm intermediate filament in mature astrocytes of the central nervous system (CNS). Over a decade ago, the value of GFAP as aprototype antigen in nervous tissue identification and as a standard marker for fundamental and applied research at an interdisciplinary level was recognized [147]. As a member of the cytoskeletal protein family, GFAP is thought to be important in regulating astrocyte motility and shape by providing structural stability to

astrocytic processes [148]. In the CNS of higher vertebrates, after injury, either as a result of trauma, disease or genetic disorders, astrocytes become reactive and responds in a typical manner, termed astrogliosis. Astrogliosis, characterized by enhanced expression of the glial fibrillary acidic protein. Thus, increases in GFAP serve as a sensitive and quantitative index of neural damage [148].

1.5.2. Glutathione reductase:

Glutathione reductase (GR) is substrate specific antioxidant enzyme that belongs to a member of pyridine-nucleotide disulfide oxidoreductase, family of flavoenzymes, which plays an essential role in catalyzing the oxidized forms of glutathione (GSSG) into reduced form (GSH) [149].

GSH is an antioxidant in glutathione metabolism, involved in most cellular and molecular functions. Moreover, GSH dependent enzymes utilizes GSH as substrate for their functions, therefore donating electron from reduced GSH itself and become reactive, these two reactive species (free radicals) forms glutathione disulfide (GSSG). Accumulating GSSG is referred to as oxidative stress and cytotoxic, hence, it is maintained at low strength comparing with GSH. Homeostasis of glutathione pool is regulated by GR [149].

1.5.3. Neuronal nitric oxide synthase:

Neuronal nitric oxide synthase (nNOS) is constitutively expressed in central and peripheral neurons and some other cell types, which is responsible for production of small signaling molecule known as nitric oxide (NO). Enzyme activity is regulated by Ca^{2+} and calmodulin[150].

Neuronal NOS has been implicated in modulating physiological functions such as learning, memory, and neurogenesis [151]. In the central

nervous system, nNOS mediates long-term regulation of synaptic transmission (long-term potentiation, long-term inhibition) [152]; whereas there is no evidence for an involvement of nNOS-derived NO in the neurotransmission [153].

High levels of NO can inhibit mitochondrial respiration, which leads to energy depletion, in two ways: (i) reversible inhibition at cytochrome oxidase, and (ii) irreversible inhibition of cytochrome oxidase, ATP synthase, creatine kinase, and aconitase by generating a peroxynitrite [154]. Abnormal NO signaling is likely to contribute to a variety of neurodegenerative pathologies such as excitotoxicity following stroke, multiple sclerosis, Alzheimer's, and Parkinson's diseases[155] Hyperactive nNOS, stimulated by massive Ca^{2+} influx into neuronal cells, has been implicated in N-methyl-D-aspartate receptor mediated neuronal death in cerebrovascular stroke. Under those conditions, NO can contribute to excitotoxicity, probably via peroxynitrite production, that can cause oxidative damage, nitration, and S-nitrosylation of biomolecules including proteins, lipids, and DNA [156].

1.6. Aim of the study:

This study was designed to:

1- Investigate the effect of different oral doses of atorvastatin on normal male adult rats via studying immunohistochemical markers in the hippocampus.

2- Investigate the effect of different oral doses of atorvastatin on male adult rats model of Alzheimer's disease induced by 3 mg/kg streptozocin intrathecally via studying immunohistochemical markers in the hippocampus.

Chapter Two

Materials and Methods

2.1. Chemicals

All chemicals and reagents were of the highest available purity and need no more purification. Specific chemicals and reagents used in this study are shown in table (2-1) with their suppliers.

Table (2-1): Chemicals, reagents and their suppliers.

Chemicals	Suppliers
DAB substrate	Cat no. ab80436, Abcam-UK.
Mouse Specifying Reagent (Complement)	Cat no. ab80436, Abcam-UK.
Goat anti-rabbit HRP Conjugate	Cat no. ab80436, Abcam-UK.
Hydrogen Peroxide Block	Cat no. ab80436, Abcam-UK.
Protein Block	Cat no. ab80436, Abcam-UK.
DAB Chromogen	Cat no. ab80436, Abcam-UK.
Streptozocin vial (500 mg).	Cat no. ab142155, Abcam-UK.
Vastor® (Atorvastatin 10 mg) tablets	Batch no. 3031, HIKMA-Jordan
Antibody Diluent	Cat no. ab64211, Abcam-UK.
Formalin (37%-40%)	SIGMA CHEMICAL co.-USA
Total cholesterol kit	Spinreact, Spain
Triglyceride kit	Spinreact, Spain
HDL-cholesterol kit	Randox laboratories, UK
Ethanol (99%)	Scharlau-Spain
Xylene	Biosolve-France
Diethyl Ether	QualiKems-India
Paraffin wax (5 kilos)	Medite-USA

Hematoxylin crystals	SyrBio-Switzerland
Eosin crystals	SyrBio-Switzerland
Tween 20	SCRC-China
DPX mounting medium	SyrBio-Switzerland
Positively charged microscope slides	Fisherbrand Superfrost- USA

The primary immunohistochemical kits used in the study with their description showed in the table (2-2).

Table (2-1): types of primary kits.

Name	Description	Cat no.	Company
Anti Fibrillary Glial Protein antibody (GFAP antibody)	Rabbit polyclonal to GFAP –Astrocyte Marker	ab48050	Abcam-UK
Anti-Glutathione Reductase antibody	Rabbit polyclonal	ab16801	Abcam-UK
Anti- neuronal nitric oxide synthase antibody (nNOS antibody)	Rabbit polyclonal	ab106417	Abcam-UK

2.2. Instruments:

Instruments used in this study are summarized in table (2-3) with their suppliers.

Table (2-3): instruments used in the study with their suppliers.

Instruments	suppliers
Mettler H54 A.R. Microbalance	Karl Kolb-Germany
Rotary Microtome	KEDEE, KD-1508A-Japan
Binocular light microscope	MED, SEEUCO-China
oven	Memmert-Germany
Microwave	Haier-USA
Centrifuge	Hettich Universal-Germany

2.3. Animals and study design:

Fourty eight adult male Wistar rats (weighing 200–250 gm) were used in the experiment. They were obtained from the animal house (at Department of Pharmacology & Toxicology, College of Pharmacy/ The University of Mustansiriya). Animals were divided into 8 groups randomly each group contains 6 animals as follow:

Group 1: Animals were administered saline orally for 30 days and serve as a control group.

Group2: Animals were administered intrathecal injection of 3 mg/kg Streptozocin as a single dose and saline orally for 30 days.

Group 3: Animals were administered intrathecal injection of 3mg/kg Streptozocin as a single dose. At the same day, 5 mg/kg/day Atorvastatin were administered in the form of oral suspension, by using oral gavage tube, continued for 30 days.

Group 4: Animals were administered intrathecal injection of 3mg/kg Streptozocin as a single dose. At the same day, 10 mg/kg/day Atorvastatin were administered in the form of oral suspension, by using oral gavage tube, continued for 30 days.

Group 5: Animals were administered intrathecal injection of 3 mg/kg Streptozocin as a single dose. At the same day, 20 mg/kg/day Atorvastatin were administered in the form of oral suspension, by using oral gavage tube, continued for 30 days.

Group 6: Animals were administered 5 mg/kg/day Atorvastatin in the form of oral suspension by using oral gavage tube for 30 days.

Group 7: Animals were administered 10 mg/kg/day Atorvastatin in the form of oral suspension by using oral gavage tube for 30 days.

Group 8: Animals were administered 20 mg/kg/day Atorvastatin in the form of oral suspension by using oral gavage tube for 30 days.

All groups were kept in plastic cages of (20x25x35 cm) dimension. Six animals per cage were kept under controlled conditions of temperature of (22 ± 1^0C) with light schedule of 12-12 hours light/dark cycles and the animal house was provided with an air vacuum. Tap water and foods in the form of pellets were accessible freely to the animals. The animals were kept for 2 weeks in the mentioned conditions before starting treatment to be adapted to the environment of the animal house.

Streptozocin dissolved in sodium citrate buffer at PH (4.5) and the powder was weighed using the Mettler H54 A.R. Microbalance (Karl Kolb, West Germany). Each 3 mg dissolved in 1ml of sodium citrate buffer, and administered intrathecally by using ordinary syringe under sterilized condition, while each 1 tablet of atorvastatin (10 mg) was grinded by mortar and pistol and weighed. After that, the powder was dissolved in 10 ml of distilled water to obtain an oral suspension of (1mg/1ml).

Direct instillation of a compound into the stomach or lower esophagus (gavage) of a conscious rat is by far the most accurate method to administer drugs into the gastrointestinal tract. However, because this technique is performed in a conscious rat, iatrogenic injury to the esophagus and pulmonary aspiration are possible sequelae.

Using adequate manual restraint, the rat was held in a vertical position with its nose aimed toward the ceiling so as to form a straight line between the rat's mouth and stomach. Next, the gavage needle was gently inserted into the oral cavity through the left diastema and passed along the roof of the oral cavity toward the ramus of the right mandible. As the animal swallows, the instrument was advanced down into the esophagus. Finally, once the needle was advanced to the appropriate depth, the solution slowly infused by depressing the plunger of the syringe (fig. 2-1).

Figure (2-1): administration of Atorvastatin by oral gavage tube.

All animals in this study were dissected under anesthesia using diethyl ether at day 31. With the animals under anesthesia and the heart still beating, collection of blood samples were done and the head was decapitated for extraction of the brain. The blood was collected for measuring cholesterol, low density lipoprotein (LDL), triglyceride (TG) and high density lipoprotein (HDL).

2.4. Methods:

2.4.1. Obtaining of the brain:

The scalp was dissected free from the calvaria which were opened by a scissors or a bone cutter, starting at the interorbital region, and proceeding towards interaural line on each side. The dissected calvaria was elevated and removed. The whole brain was delivered by transecting it at the spino-

medullary junction then divided it into two halves, each one was processed separately (fig. 2-2).

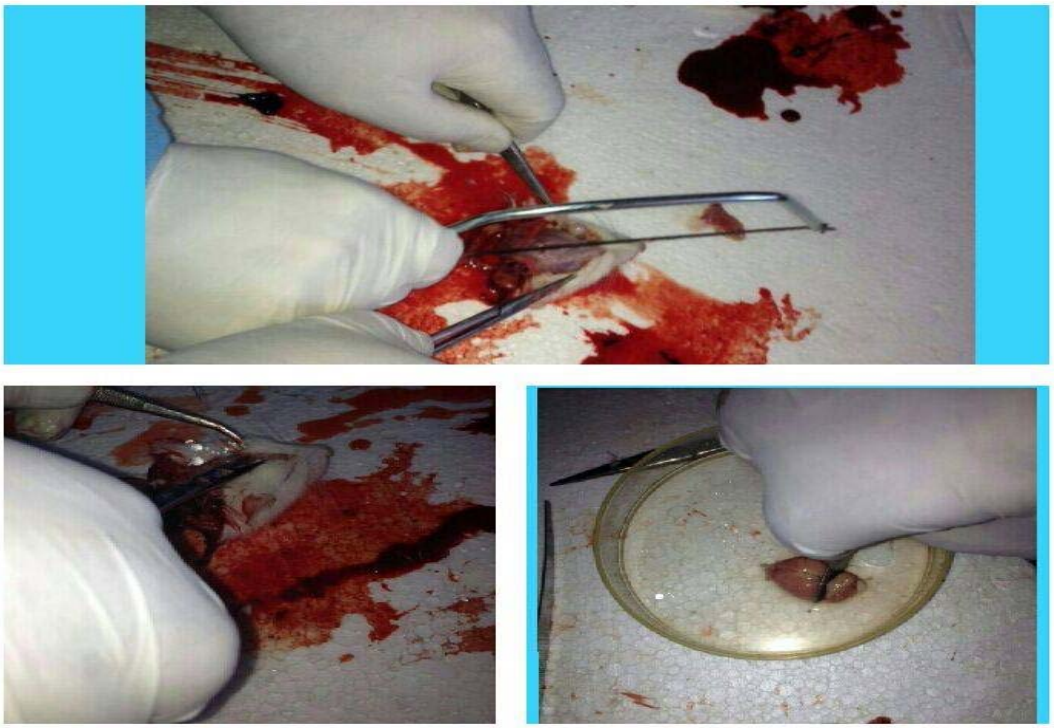

Figure (2-2): Obtaining of the rat's brain.

2.4.2. Preparation for histological study:

The tissues (brain) were processed according to Bancroft and Stevens [146] as follows:

1. Fixation:

Immediately after separation, the specimens were fixed individually in 10 % formalin buffer solution for 24 hours at room temperature. Prolong fixation usually yields less accurate results (false negative).

2. Dehydration:

The tissues were removed from the fixative, and immersed in graded progressively increasing concentration alcohol baths at room temperature as follows:

a- two changes of 70% ethanol, each change for 2 hours.

b- 90% ethanol for 2 hours.

c-two changes of absolute ethanol, each change for 2 hours.

3. Clearing:

After dehydration, the tissues were kept in xylene for 1 hour, and then second change was done with xylene at 60^0C in the oven for another 1 hour to remove alcohol and give the tissues some degree of transparency.

4. Wax impregnation and Embedding:

Specimens were passed through baths of molten paraffin wax (56^0C melting point) for two changes, 3 hours for each in an embedding oven to remove the clearing agent. Embedding was done in special stainless steal containers (molds); the specimens were transferred from the paraffin bath to the embedding molds. When the wax surface (in the mold) was solidified, the mold was putted in cold ice water. All molds were routinely labeled. Rapid cooling given the wax better properties and reduced the wax crystals. The paraffin wax was used from Medite, USA.

5. Sections cutting:

Sectioning was done by rotatory microtome (KEDEE, KD-1508A) with disposable blades. After appropriate trimming, the section thickness was set to 5 μm and several sections were taken from each block. Sectioning started at medial surface of cerebral hemisphere and proceeded laterally

towards the temporal pole. Sections were floated on the water bath for 30 seconds to flatten the tissue section then the sections were placed onto slides.

2.4.3. Staining for general morphology:

After tissues embedding and sectioning, method of Hematoxylin and eosin staining for formalin fixed-paraffin embedded tissue was used as following [146]:

1-Deparaffinization: was done by leaving sectioned slides in oven at $70C^0$ for two hours. Sections were then dipped in 2 successive changes of xylene:

a-Pre-warmed (55^0C) xylene for 5 minutes.

b-Xylene (at room temperature 20-25 ^{0}C) for 2 minutes.

2-Hydration: This was done by immersion of the tissue slides in descending concentration of ethanol and distilled water as follows:

100% two changes, 95% one change and 70% one change (one minute for each change). Then washed under running tap water for 10 minutes.

3-Staining: hematoxylin and eosin stain was used as following:

a-stained by Harri's Hematoxylin for 3 minutes *(*3 minutes yield optimal staining, longer than that, background starts to show up).

b-wash well in running tap water until for 5 minutes or less.
c-treated with acid alcohol 3-10 quick dips.
d-washed in tap water very briefly.
e-dipped in ammonia water until become bright blue.
f-tissue sections were stained with eosin for 40 seconds.
g-dehydrated in an increasing grades of ethanol as following:
90% ethanol for 1 minute (twice), 99% ethanol for 1 minute (twice).

h-clearing by two changes of xylene (2 minutes each).
i-mounting in DPX and covered with cover slips.

2.4.4. Immunohistochemistry for detection of neuronal nitric oxide synthase, glial fibrillary acidic protein and glutathione reductase expression in paraffin-embedded sections:

2.4.4.1. Principles of the procedure:

A biotin free-horseradish peroxidase Conjugate, 3-3'diaminobenzidine HRP/DAB system is intended for use with primary antibody from rabbit or mouse for the qualitative identification of antigens by light microscopy and immunohistochemistry in formalin fixed and paraffin-embedded tissues. This system is a sensitive and versatile immunohistochemistry procedure. Endogenous peroxidase activity is quenched by incubating the specimen with 3% hydrogen peroxide. The specimen is then incubated with an appropriately characterized and diluted rabbit or mouse primary antibody, followed by sequential incubation with a horseradish peroxidase conjugate and complement antibody. Staining is completed after incubation with the substrate-chromogen (3-3' diaminobenzidine) (DAB) which result in a brown-colored precipitate at the antigen site and the section may be mounted with any permanent mounting media. A primary antibody reacts with an antigen. A biotin free secondary antibody then reacts with the primary antibody [146].

2.4.4.2. Fixation

Tissue blocks immersed in (10%) neutral buffered formalin for (24 hours) at room temperature were processed for paraffin sectioning. This

fixation protocol was recommended in order not to affect the tissue binding sites of the immunohistochemical markers used in the study.

Serial sections of (5 μm) thickness were obtained in the sagittal plane, using rotary microtome (KEDEE, KD-1508A). Sectioning started at medial side and proceeded laterally towards the temporal pole. When reached the hippocampal region, two consecutive serial sections were obtained at appropriate intervals, for immunohistochemistry.

2.4.4.3. Preparation of tissue sections and reagents:

1-hematoxylin (5 gm) was dissolved in 50 ml of alcohol; the 100 gm potassium alum in the water by the aid of heat. Then removed from heat and the two solutions mixed and; boiled for less than 1 minute with stirring (bringing to boiling should be rapid as soon as possible). Then removed from heat and the mercuric oxide (2.5 gm) added slowly. Re-heated to a simmer until it becomes dark purple, removed from heat and the vessel plunged into a basin of cold water till cooled. 2-4 ml of glacial acetic acid per 100 ml added (to increase the precision of nuclear stain). Filter before use.

2-paraffin embedded sections were cut into 5 micron thick. Placed on Fisherbrand Superfrost/Plus slides and left overnight at room temperature to dry.

3-Formalin Solution (10% buffered neutral) was prepared, as recommended by the manufacturer kit's leaflet, by adding 900 ml of distilled water to 100 ml of formaldehyde (37-40%) with 4 gm. and 6.5 gm. of sodium di-hydrogen phosphate (monobasic) and sodium phosphate dibasic (anhydrous) respectively, then mix well to be used in the fixation process.

4- Phosphate Buffer Saline (PBS) solution was prepared by mix 8, 0.2, 0.2 and 0.92 gm of NaCl, KCl, KH2PO4 and Na2HPO4 powder respectively. Then adding 1000 ml of deionized water to the powder and mix well. Store unused buffer at 2-8 ^{0}C. Discard buffer if cloudy in appearance.

5- Sodium Citrate Buffer (for antigen retrieval): Dissolve Tri-sodium citrate (dihydrate) (2.94 g) in 1000 ml of distilled water. Mix well to dissolve and adjust pH to 6.0 with 1N HCl. Add 0.5 ml of Tween 20 and mix well. Store at room temperature for 3 months or at 4^{0}C for longer storage.

6-substrate-chromogen solution: Add 1 drop (or 20 microliter) of the DAB chromogen per 1.5 ml of substrate buffer and mix well. This solution should be protected from light and used within one hour.

7-primary antibody was diluted in antibody diluent by adding 1µl of it per 100µl of diluents as follows:

a-5 µg/ml for Anti-nNOS (neuronal) antibody.

b-2 µg/ml for Anti-Glutathione Reductase antibody.

c-0.5 µg/ml for Anti-GFAP antibody – Astrocyte.

8-Absolute ethanol was diluted in distilled water to prepare 95%, 90% and 70% concentrations of alcohol.

2.4.4.4. Immunohistochemistry procedure: involve the following steps [146]:

1-serial tissue sections were cut 5 micron thick and floated in protein free water bath.

2-the sections were positioned on positively charged slides carefully.

3-Dewaxing: paraffin embedded sections were placed inside a hot air oven at 65^0C overnight, then dipped in xylene and ethanol containing jars in the following order:

a- Xylene for 3 minutes.
b- Fresh xylene for 3 minutes.
c- 1: 1 xylene to absolute ethanol for 3 minutes.
d- Absolute ethanol for 3 minutes.
e- Fresh absolute ethanol for 3 minutes.
f- Ethanol (95%) for 3 minutes.
g- Ethanol (70%) for 3 minutes.

4-Slides were washed in distilled water. Keep the slides in the water until ready to perform antigen retrieval.

5-Antigen retrieval: Heat pretreatment in Sodium Citrate buffer (PH 6) at 100 ^{0}C for 20 minutes.

6-100 µl of Hydrogen peroxidase block solution was placed onto the section and incubated for 10 minutes in a humid chamber at room temperature (20-25 ^{0}C). Then slides were drained and blotted gently.

7- Slides were rinsed 2 x 1min in PBS with gentle agitation, then drained and wipe around the sections with tissue paper gently.

8-100 µl of a protein-blocking reagent was placed onto the section and incubated for 10 minutes in a humid chamber at room temperature. Then slides were drained and blotted gently.

9-100 µl of diluted primary antibody was placed onto the section and incubated for 1 hour at 37 ^{0}C in a humid chamber. After incubation, the slides were drained and blotted gently.

10- Slides were rinsed 2 x 1min in PBS with gentle agitation, then drained and wipe around the sections with tissue paper gently.

11-100 µl of secondary antibody, HRP (horseradish peroxidase) conjugate, was placed onto the section and incubated for 10 minutes at 37^0C in a humid chamber. Slides were drained and blotted gently.

12- Slides were rinsed 2 x 1min in PBS with gentle agitation, then drained and wipe around the sections with tissue paper gently.

13-100 µl of complement solution was placed onto the section and incubated for 15 minutes at 37^0C in a humid chamber. Slides were drained and blotted gently.

14- Slides were rinsed 2 x 1min in PBS with gentle agitation, then drained and wipe around the sections with tissue paper gently.

15-100 µl of substrate-chromogen solution was placed onto the section and incubated for 10 minutes at 37^0C in a humid chamber.

16- Slides were washed with distilled water from a washing bottle for few minutes, then drained and blotted gently.

17-100 µl of counterstain (Hematoxylin) was placed onto the section and incubated for 3 minutes at room temperature. Slides were drained and blotted gently.

18-slides were washed in distilled water then dehydrated by placing them in ethanol and xylene in the following order:

a- 70% ethanol for 3 minutes.
b- 95% ethanol for 3 minutes.
c- Absolute ethanol for 3 minutes.

d- 1:1 Xylene to absolute ethanol for 3 minutes.

e- Xylene for 3 minutes.

f- Fresh xylene for 3 minutes.

19- A drop of mounting medium (DPX) was placed onto the xylene-wet section by using a xylene-moist cotton swab and the section was quickly covered with a cover slip. Slides were let to dry.

2.5. Examination and quantification (scoring):

Slides were examined with high magnification power to identify semi-quantitatively partial and complete stained tissues that defined as positive for the markers.

2.5.1. Glial Fibrillary Acidic Protein (GFAP):

Scoring of GFAP expression was done for percentage of staining intensity per field as following [157]:

0 = none

1 =< 5%

2 =5-25%

3 = 25-75%

4 =75-100%

2.5.2. Glutathione Reductase (GR):

An immunohistochemical based scoring system was utilized for analyses of GR as percentage of positive stained cells per field in a blind fashion and the scores calculated as following [158]:

0 = no stain %

1 = <15%

2 = 15-25%

3 = 25-50%

4 = 50-75%

5 = >75-100%

2.5.3. Neuronal Nitric Oxide Synthase (nNos):

The immunostaining was graded in five classes according to the percentage of stained tissue [159]:

0= when the staining was absent.

1=when the percentage of stained tissue varied from 1% -25%.

2= when the percentage of stained tissue varied from 26%- 50%.

3= when the percentage of stained tissue varied from 51%-75%.

4= when the percentage of stained tissue was superior to 75%.

2.6. Measurement of serum lipid profile:

2.6.1. Serum cholesterol determination:

Serum cholesterol level was determined after enzymatic hydrolysis by cholesterol esterase (CHE) and oxidation by cholesterol oxidase (CHOD) using a ready-made kit [160]. The resulted hydrogen peroxide (H_2O_2) then reacts with 4-aminophenazone in the presence of phenol and peroxidase (POD) to form a colored complex quinoneimine, according to the following equations:

$$\text{Cholesterol esters} + H_2O \xrightarrow{\textbf{CHE}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} 4 - \text{Cholestenona} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + 4 - \text{Aminophenazone} \xrightarrow{\text{POD}} \text{Quinonimine} + 4H_2O_2$$

The intensity of the pinkish color formed was measured at 500 nm and serum cholesterol concentration calculated from the equation:

$$\text{TC concentration (mg/dl)} = \{\frac{\text{Absrbance of the sample}}{\text{Absorbance of the standard}}\} \times 200 \text{ (Standard conc.)}$$

2.6.2. Serum triglyceride (TG) determination:

Serum triglyceride was measured using a ready-made kit according to the method of Buccolo *et al*. [161], which is based on the incubation of sample triglyceride with lipoprotein lipase (LPL); liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate (G3P) is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2).

In the last reaction, hydrogen peroxide (H_2O_2) reacts with 4-aminophenazone (4-AP) and p-chlorophenol in presence of peroxidase (POD) to give a red colored dye:

$$\text{Triglycerides} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{G3P} + \text{ADP}$$

$$\text{G3P} + O_2 \xrightarrow{\text{GPO}} \text{DAP} + H_2O_2$$

$$H_2O_2 + 4-\text{AP} + \text{P}-\text{Chlorophenol} \xrightarrow{\text{POD}} \text{Quinone} + H_2O$$

The intensity of the color formed was measured spectrophotometrically at 505 nm and serum triglyceride concentration calculated from the equation:

$$\text{TG conc. (mg/dL)} = \{\frac{\text{Absorbance of the sample}}{\text{Absorbance of the standard}}\} \times 200 \text{ (standard conc.)}$$

2.6.3. Serum low density lipoprotein determination:

The concentration of serum low density lipoprotein (LDL) was calculated using Friedewald formula below [162]; the results were expressed in mg/dL.

$$\text{LDL-c= Total cholesterol}-\text{HDL cholesterol} - \left[\frac{\text{TG}}{5}\right]$$

2.6.4. Serum high density lipoprotein (HDL) determination:

Serum high density lipoprotein-cholesterol (HDL) was measured using a ready-made kit for this purpose [163]. The principle of this test based on precipitation of low density lipoproteins fractions by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in HDL lipoprotein fraction, which remains in the supernatants, was determined and measured at 500 nm wave length and HDL concentration obtained from the equation:

$$\text{HDL conc. (mg/dL)} = \left\{\frac{\text{Absorbance of the sample}}{\text{Absorbance of the standard}}\right\} \times \text{(standard conc.)}$$

2.7. Statistical analysis

Data were analyzed by using One Way Analysis of Variance (ANOVA). Statistical evaluation of the data was performed by the nonparametric Kruskal–Wallis test and comparisons among groups were submitted according to Dunn's Multiple Comparison Test using the program GraphPad Prism 5.0. Whereas the parameters of lipid profile were evaluated by Student t-test to assess significant difference among means. *P-values* less than 0.05 were considered significant for all data presented in the results.

Chapter Three

Results

3.1. Effects of different doses of Atorvastatin on Glial Fibrillary Acidic Protein (GFAP):

The descriptive statistics, which represent the mean, median, minimum value and maximum value for the score of GFAP expression in groups treated with atorvastatin, are summarized in table (3-1). Analysis of data statistically by Kruskal-Wallis test revealed a significant difference ($p<0.05$) among the groups (table 3-2). Dunn's Multiple Comparison Test was done and the table (3-3) shows that there is a non-significant change ($p>0.05$) in the score of the GFAP expression of the control group and the group of rats that administered 5 mg/kg of atorvastatin orally. Moreover, table (3-3) demonstrated that there is another non-significant decrease ($p>0.05$) in the group of rats treated with 10 mg/kg of atorvastatin (fig.3-4) when compared with the control group (fig. 3-2). There were no signs of significancy ($p>0.05$) when the 10 mg/kg atorvastatin group was put against the 5 mg/kg and 20 mg/kg atorvastatin groups. On the other hand, a significant decrease ($p<0.05$) in the 20 mg/kg atorvastatin treated group (fig. 3-5) was noticed when compared to the other two groups, the 5 mg/kg atorvastatin (fig. 3-3) and the control group. As mentioned earlier, this difference in scores is due to the difference of staining intensity of glial fibrillary acidic protein (GFAP). Figure (3-1) testifies the effects of various atorvastatin doses on that marker.

Table (3-1): descriptive statistics for the score of GFAP expression in groups treated with atorvastatin.

Groups	Sample No.	25% Percentile (Q1)	75% Percentile (Q3)	Minimum value	Maximum value	Mean± SD	Median (Q2)
Control	6	2.000	2.250	2.000	3.000	2.167± 0.408	2.000
Atorvastatin 5mg/kg	6	2.000	2.250	2.000	3.000	2.167± 0.408	2.000
Atorvastatin 10mg/kg	6	1.750	2.000	1.000	2.000	1.833± 0.408	2.000
Atorvastatin 20mg/kg	6	1.000	2.000	1.000	2.000	1.333± 0.516	1.000

*Q: quartile.

Table (3-2): Kruskal-Wallis test for the score of GFAP expression in groups treated with atorvastatin.

Test	Statistical value	P value
Kruskal-Wallis statistic	10.03	0.0183

Table (3-3): Effects of different doses of atorvastatin on glial fibrillary acidic protein.

Dunn's Multiple Comparison Test	Difference in rank sum	Significancy
Control vsAtorvastatin.5mg	0.0000	NS
Control vsAtorvastatin 10mg	3.417	NS
Control vs Atorvastatin 20mg	8.917	S
Atorvastatin.5mg vs Atorvastatin 10mg	3.417	NS
Atorvastatin.5mg vs Atorvastatin 20mg	8.917	S
Atorvastatin 10mgvsAtorvastatin 20mg	5.500	NS

S: Significant difference ($p<0.05$).

NS: non-significant ($p>0.05$).

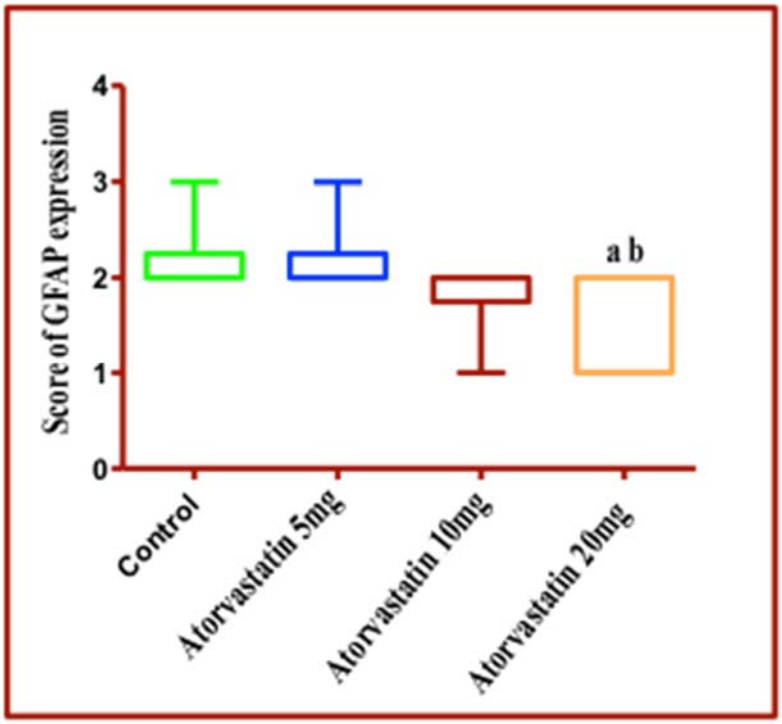

Figure (3-1): box and whisker plot showing the effects of different doses of atorvastatin on the expression of glial fibrillary acidic protein marker.

[a]: significantly different compared with the control.

[b]: significantly different compared with the Atorvastatin 5mg/kg group.

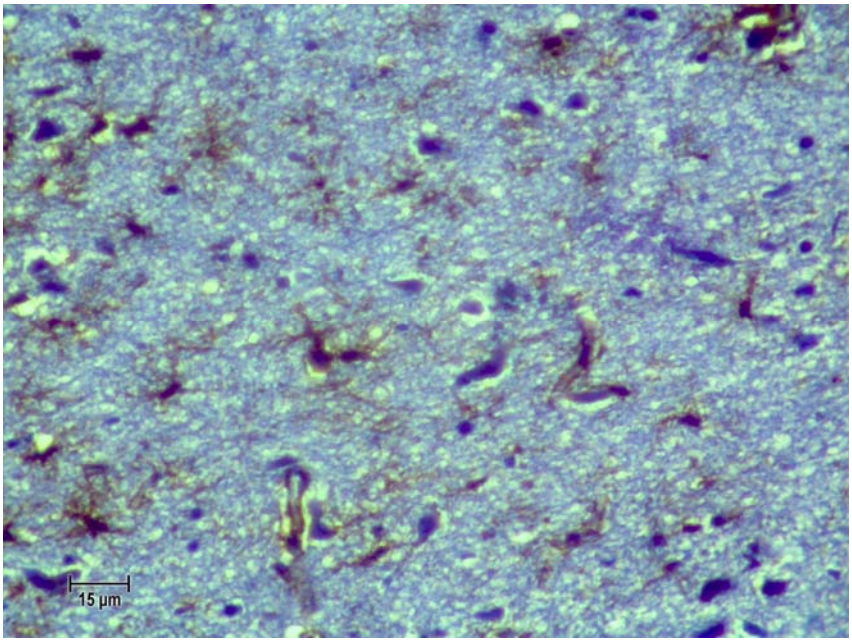

Figure (3-2): Immunohistochemical staining for hippocampus expressions of GFAP marker in the control group. (Scale bar at left lower corner represents 15 µm (400 ×)).

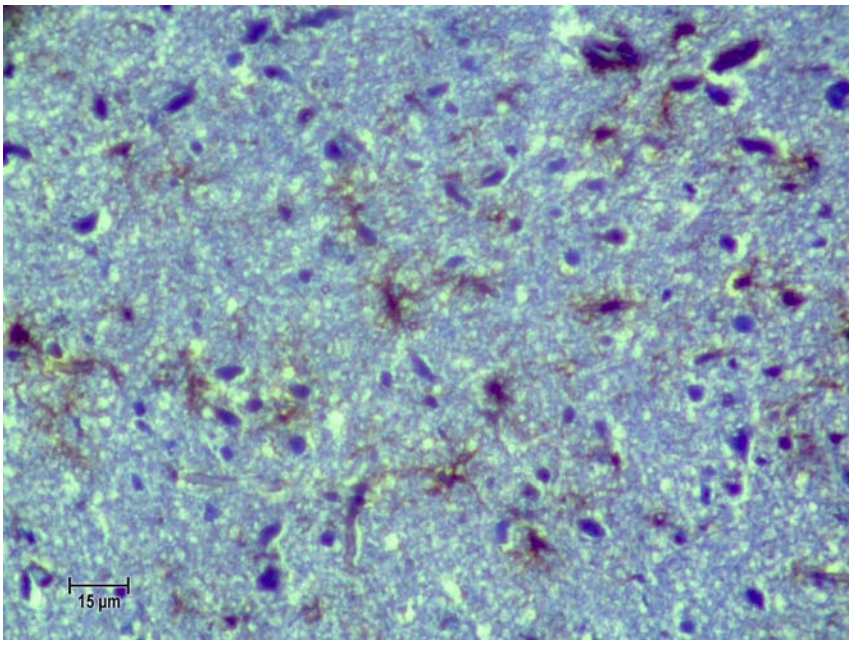

Figure (3-3): Immunohistochemical staining for hippocampus expressions of GFAP marker in the group administered 5 mg/kg atorvastatin.

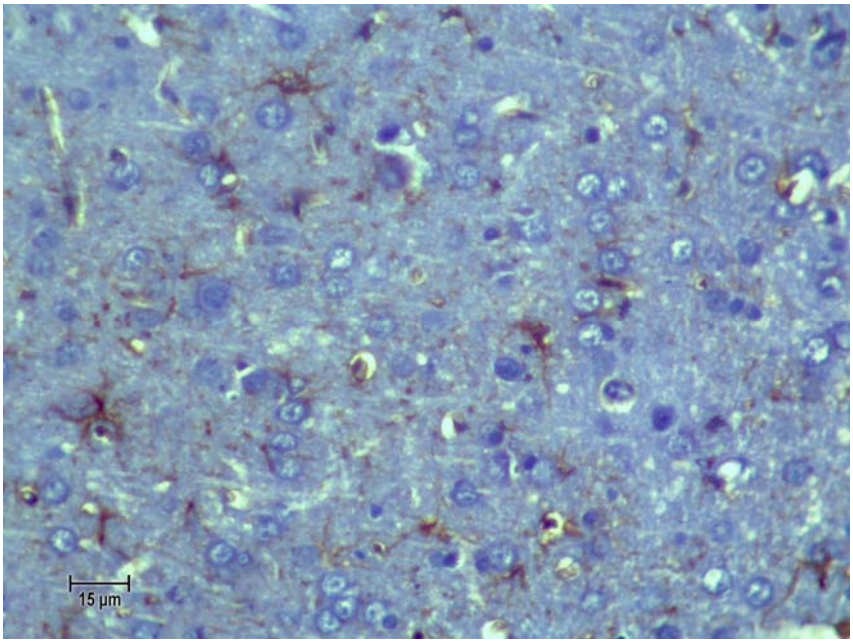

Figure (3-4): Immunohistochemical staining for hippocampus expressions of GFAP marker in the group administered 10 mg/kg atorvastatin.

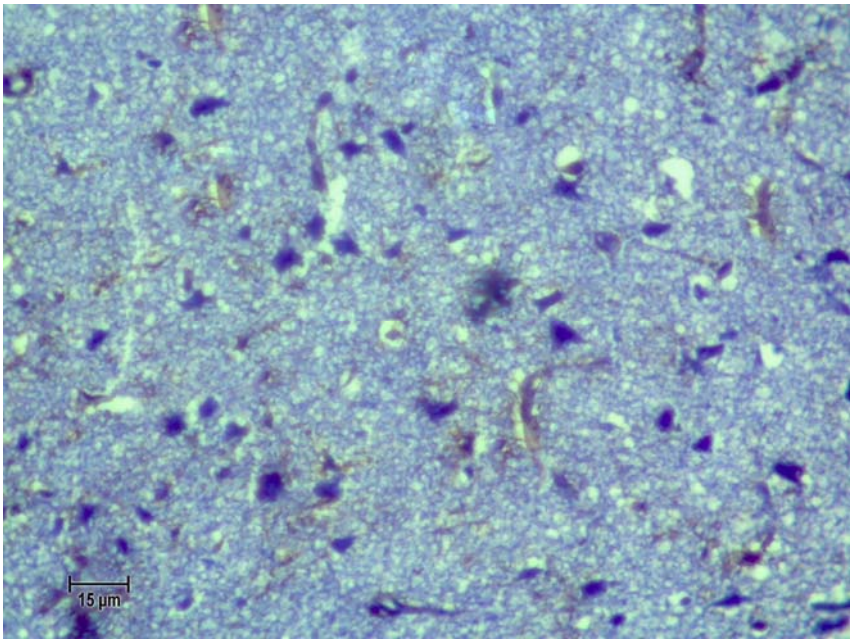

Figure (3-5): Immunohistochemical staining for hippocampus expressions of GFAP markers in the group administered 20 mg/kg atorvastatin.

3.2. Effects of streptozocin alone and various doses of atorvastatin combined with streptozocin on glial fibrillary acidic protein (GFAP):

The descriptive statistics, which represent the mean, median, minimum value and maximum value for the score of GFAP expression in the streptozocin group and groups treated with atorvastatin after administered streptozocin, are recapitulated in table (3-4). Analysis of data statistically by Kruskal-Wallis test revealed a significant difference ($p<0.05$) among the groups (table 3-5). Dunn's Multiple Comparison Test was done and the table (3-6) makes evident that there is a significant increase ($p<0.05$) in the score of the GFAP expression of group of rats administered 3mg/kg of streptozocin intrathecally (fig. 3-7) when confronted with the control. On the contrary, there was no significant difference ($p>0.05$) when the control was compared with the groups treated with 3 mg/kg of streptozocin intrathecally combined with 5 mg/kg, 10 mg/kg and 20 mg/kg of atorvastatin orally. Non significant differences were noticed when streptozocin group was opposed to both, the 5 mg/kg (fig.3-8) and the 10 mg/kg of the atorvastatin-atreptozocin (fig. 3-9) treated groups. Meanwhile, a significant decrease was observed ($p<0.05$) in the 20 mg/kg atorvastatin in addition to streptozocin group (fig. 3-10) when set side by side against the streptozocin group. All the 5 mg/kg, 10 mg/kg and 20 mg/kg atorvastatin-streptozocin treated groups showed a non significant change ($p>0.05$) when compared with each other. The figure (3-6) authenticates the results given above on glial fibrillary acidic protein.

Table (3-4): descriptive statistics for the score of GFAP expression in groups treated with streptozocin and atorvastatin.

groups	Sample No.	25% Percentile (Q1)	75% Percentile (Q3)	Minimum value	Maximum value	Mean± SD	Median (Q2)
Control	6	2.000	2.250	2.000	3.000	2.167± 0.408	2.000
Streptozocin	6	3.000	3.250	3.000	4.000	3.167± 0.408	3.000
Streptozocin.+ Atorvastatin.5mg	6	2.750	3.000	2.000	3.000	2.833± 0.408	3.000
Streptozocin.+ Atorvastatin.10mg	6	2.750	3.000	2.000	3.000	2.833± 0.408	3.000
Streptozocin.+ Atorvastatin.20mg	6	2.000	2.250	2.000	3.000	2.167± 0.408	2.000

*Q: quartile.

Table (3-5): Kruskal-Wallis test for the score of GFAP expression in groups treated with streptozocin and atorvastatin.

Test	Statistical value	P value
Kruskal-Wallis statistic	15.87	0.0032

Table (3-6): Effects of streptozocin alone and various doses of atorvastatin combined with streptozocin on glial fibrillary acidic protein.

Dunn's Multiple Comparison Test	Difference in rank sum	Significancy
Control vs Streptozocin	-13.58	S
Control vs Streptozocin.+Atorvastatin.5mg	-9.667	NS
Control vs Streptozocin.+Atorvastatin.10mg	-9.667	NS
Control vs Streptozocin.+Atorvastatin.20mg	0.0000	NS
Streptozocin. vs Streptozocin.+Atorvastatin.5mg	3.917	NS
Streptozocin. vs Streptozocin.+Atorvastatin.10mg	3.917	NS
Streptozocin. vs Streptozocin.+Atorvastatin.20mg	13.58	S
Streptozocin.+Atorvastatin.5mg vs Streptozocin.+Atorvastatin.10mg	0.0000	NS
Streptozocin.+Atorvastatin.5mg vs Streptozocin.+Atorvastatin.20mg	9.667	NS
Streptozocin.+Atorvastatin.10mg vs Streptozocin.+Atorvastatin.20mg	9.667	NS

S: Significant difference ($p<0.05$).

NS: non-significant ($p>0.05$).

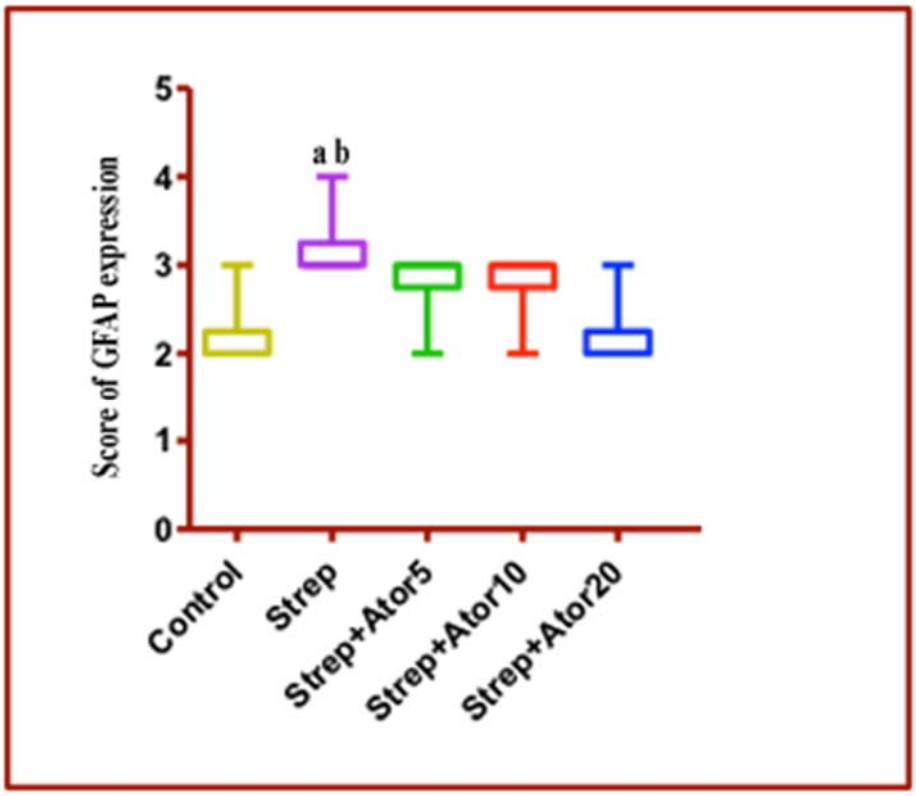

Figure (3-6): box and whisker plot showing the effects of streptozocin alone and various doses of atorvastatin combined with streptozocin on the expression of glial fibrillary acidic protein marker.

[a]: significantly different compared with the control.

[b]: significantly different compared with Streptozocin-Atorvastatin 20mg/kg group.

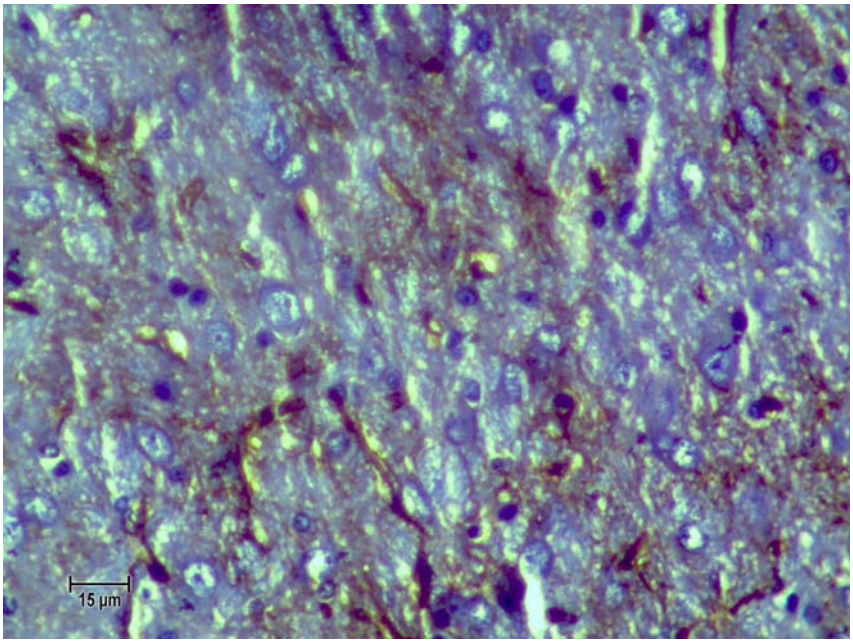

Figure (3-7): Immunohistochemical staining for hippocampus expressions of GFAP marker in the group administered 3 mg/kg streptozocin. (Scale bar at left lower corner represents 15 µm (400 ×)).

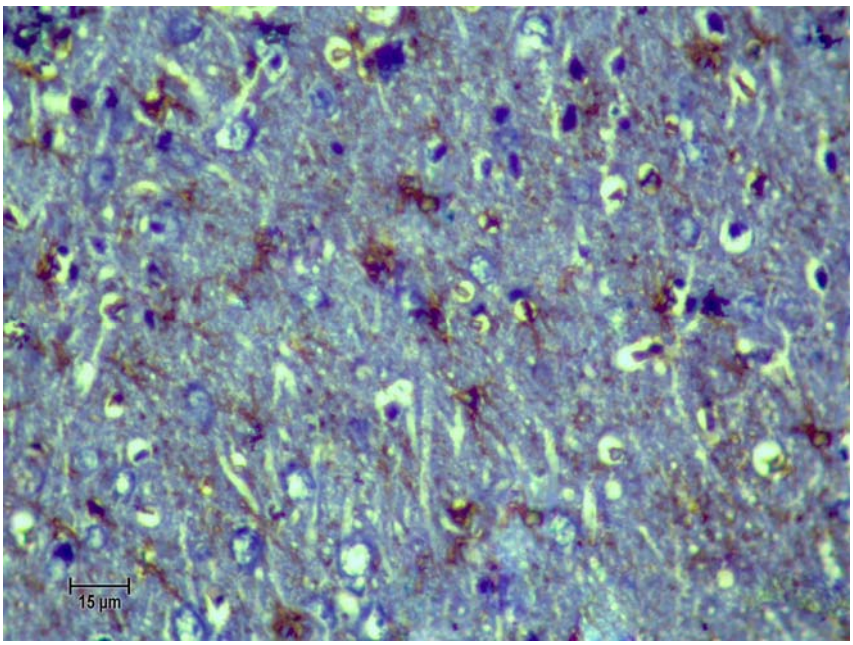

Figure (3-8): Immunohistochemical staining for hippocampus expressions of GFAP marker in the group treated with streptozocin and 5mg/kg atorvastatin.

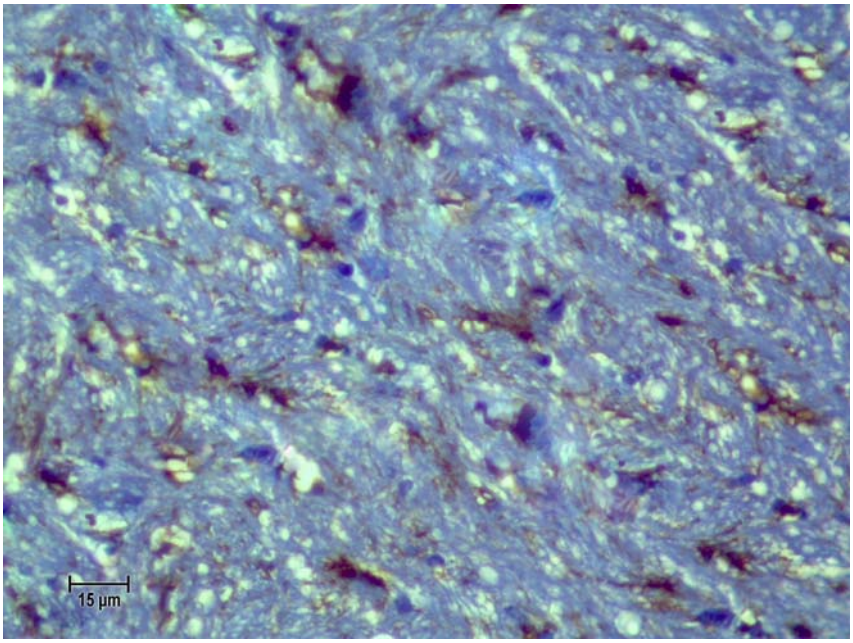

Figure (3-9): Immunohistochemical staining for hippocampus expressions of GFAP marker in the group treated with streptozocin and 10mg/kg atorvastatin.

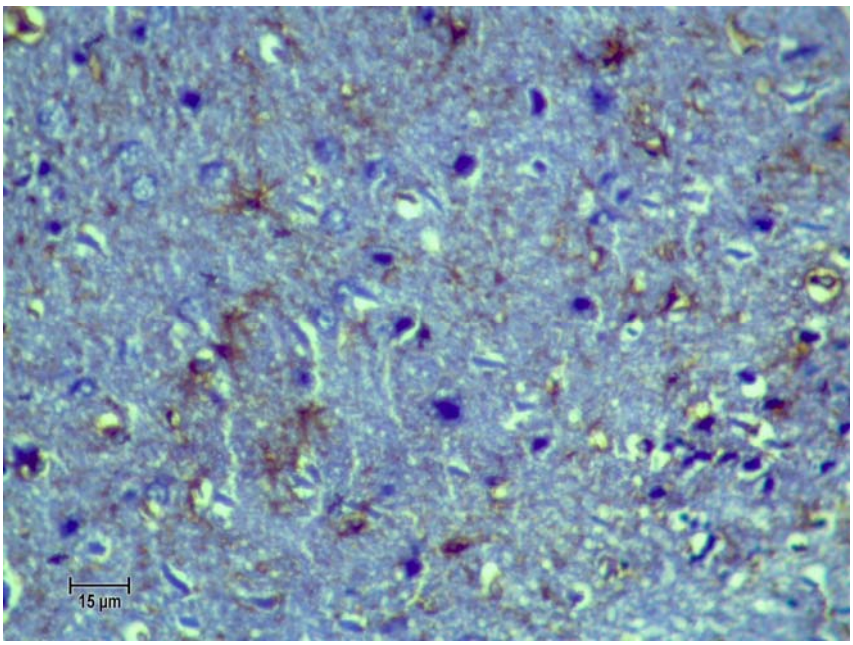

Figure (3-10): Immunohistochemical staining for hippocampus expressions of GFAP marker in the group treated with streptozocin and 20mg/kg atorvastatin.

3.3. Effects of different doses of atorvastatin on glutathione reductase (GR):

The descriptive statistics, which represent the mean, median, minimum value and maximum value for the score of GR expression in groups treated with atorvastatin, are summarized in table (3-7). Analysis of data statistically by Kruskal-Wallis test showed a significant difference ($p<0.05$) among the groups (table 3-8). Dunn's Multiple Comparison Test was done and the table (3-9) shows that there is a non significant change ($p>0.05$) in the score of the GR expression between the control group (fig. 3-12) and the group of rats treated with 5 mg/kg atorvastatin (fig. 3-13) orally. Furthermore, there is another non significant increase ($p>0.05$) in the group of rats administered 10 mg/kg atorvastatin (fig. 3-14) when compared with the control. On the other hand, table (3-9) demonstrated that a significant increase ($p<0.05$) was shown in the atorvastatin 20 mg/kg treated group (fig. 3-15) when compared to the control group.

All the 5 mg/kg, 10 mg/kg and 20 mg/kg atorvastatin groups showed a non significant change ($p>0.05$) when set side by side with each other. The figure (3-11) demonstrates the effects of different doses of atorvastatin on glutathione reductase.

Table (3-7): descriptive statistics for the score of GR expression in groups treated with atorvastatin.

groups	Sample No.	25% Percentile (Q1)	75% Percentile (Q3)	Minimum value	Maximum value	Mean± SD	Median (Q2)
Control	6	3.750	4.000	3.000	4.000	3.833± 0.408	4.000
Atorvastatin 5mg/kg	6	4.000	4.250	4.000	5.000	4.167± 0.408	4.000
Atorvastatin 10mg/kg	6	4.000	4.250	4.000	5.000	4.167± 0.408	4.000
Atorvastatin 20mg/kg	6	4.750	5.000	4.000	5.000	4.833± 0.408	5.000

*Q: quartile.

Table (3-8): Kruskal-Wallis test for the score of GR expression in groups treated with atorvastatin.

Test	Statistical value	P value
Kruskal-Wallis statistic	11.50	0.0093

Table (3-9): The Effects of various doses of atorvastatin on glutathione reductase.

Dunn's Multiple Comparison Test	Difference in rank sum	Significancy
Control vs Atorvastatin.5mg	-3.333	NS
Control vs Atorvastatin.10mg	-3.333	NS
Control vs Atorvastatin.20	-11.00	S
Atorvastatin.5 vs Atorvastatin.10	0.000	NS
Atorvastatin.5 vs Atorvastatin.20	-7.667	NS
Atorvastatin.10 vs Atorvastatin.20	-7.667	NS

S: Significant difference ($p<0.05$).

NS: non-significant ($p>0.05$).

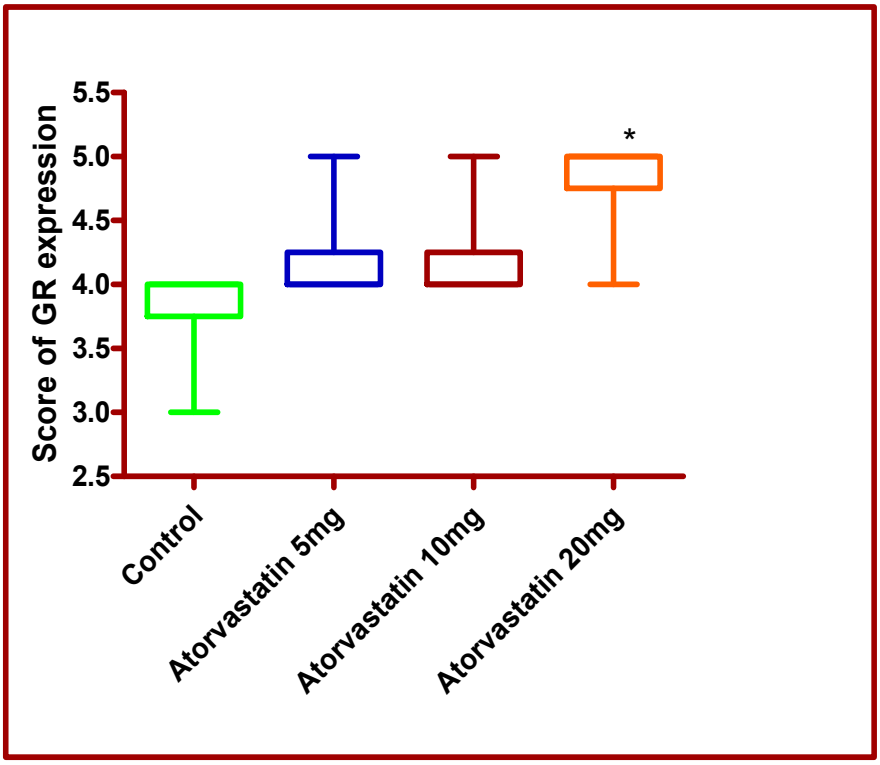

Figure (3-11): box and whisker plot showing the effects of different doses of atorvastatin on the expression of glutathione reductase marker.

*: **significantly different compared with the control.**

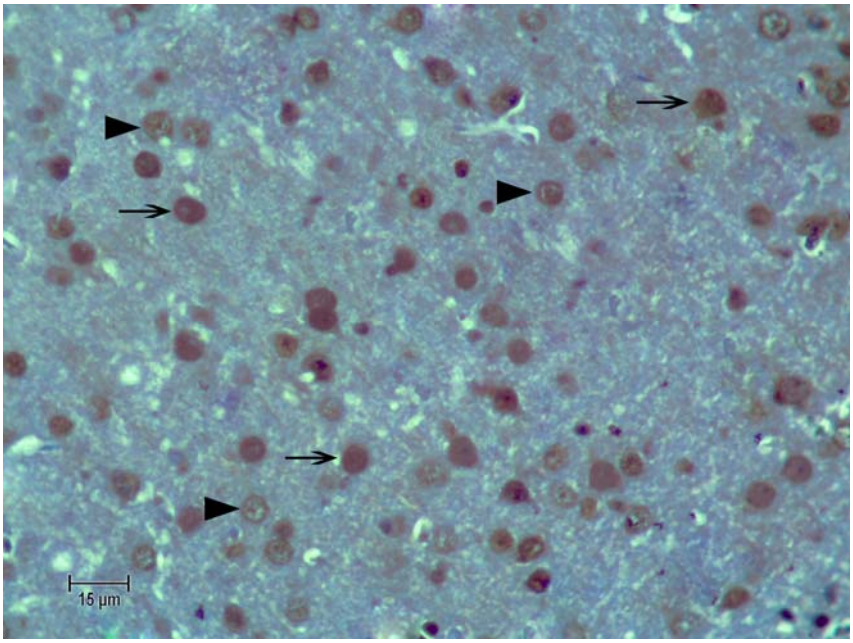

Figure (3-12): Immunohistochemical staining for hippocampus expressions of GR marker in the control. (Arrows represent positively stained cells while heads of arrows represent the negative one**.** Scale bar at left lower corner represents 15 µm (400 ×)).

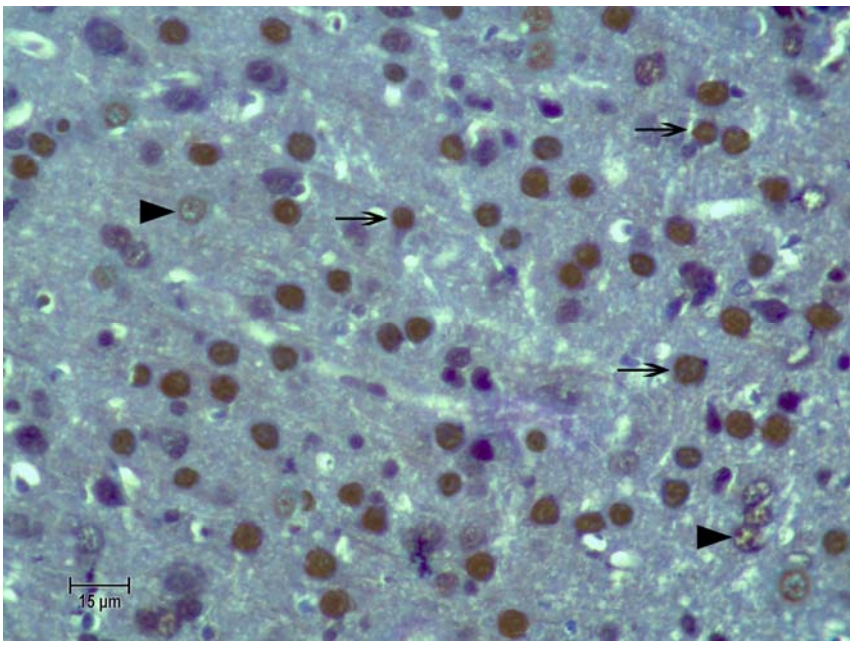

Figure (3-13): Immunohistochemical staining for hippocampus expressions of GR marker in the group administered 5 mg/kg atorvastatin. (Arrows represent positively stained cells while heads of arrows represent the negative one**).**

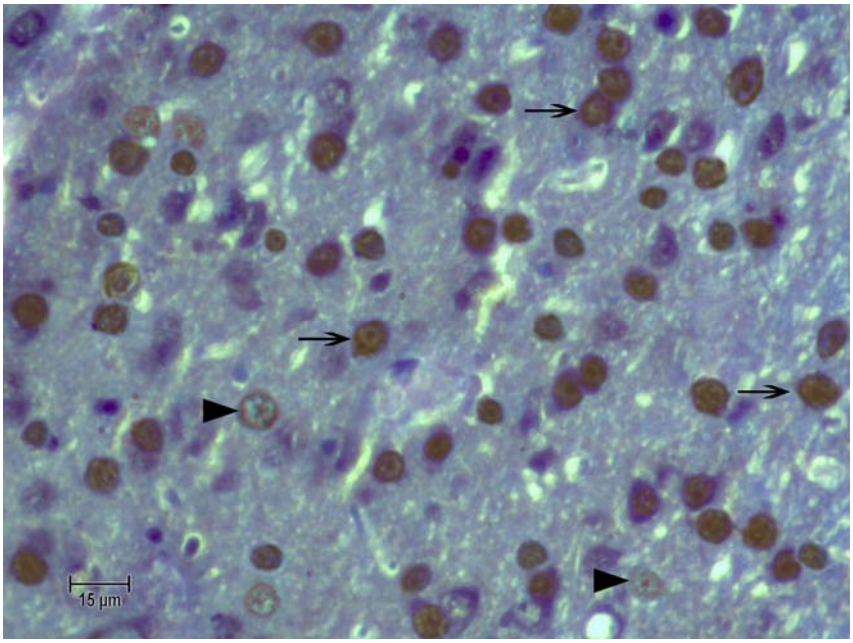

Figure (3-14): Immunohistochemical staining for hippocampus expressions of GR marker in the group administered 10 mg/kg atorvastatin. (Arrows represent positively stained cells while heads of arrows represent the negative one**).**

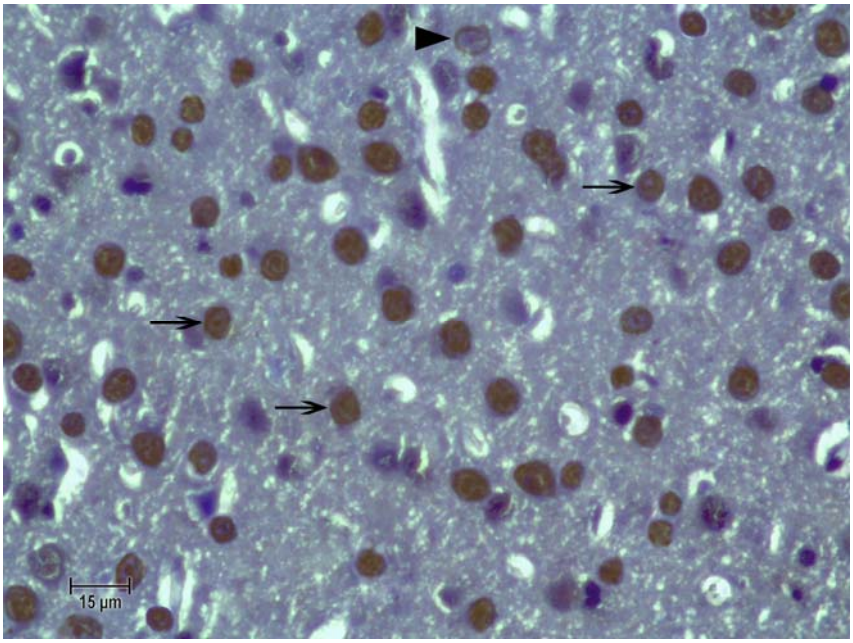

Figure (3-15): Immunohistochemical staining for hippocampus expressions of GR marker in the group administered 20 mg/kg atorvastatin. (Arrows represent positively stained cells while heads of arrows represent the negative one**).**

3.4. Effects of multiple doses of atorvastatin combined with streptozocin and streptozocin alone on glutathione reductase (GR)

The descriptive statistics, which represent the mean, median, minimum value and maximum value for the score of GR expression in the streptozocin group and groups treated with atorvastatin orally after administered streptozocin intrathecally, are recapitulated in table (3-10). Analysis of data statistically by Kruskal-Wallis test revealed a significant difference ($p<0.05$) among the groups (table 3-11). Dunn's Multiple Comparison Test was done and the table (3-12) makes certain that there is a significant decrease ($p<0.05$) in the score of the GR expression of the group administered 3 mg/kg of streptozocin intrathecally (fig. 3-17) when compared with the control. On the other side, there was no significant change ($p>0.05$) when the control was put against the groups who took 3 mg/kg of streptozocin intrathecally combined with 5 mg/kg, 10 mg/kg and 20 mg/kg of atorvastatin orally. Table (3-12) demonstrated that a significant decrease ($p<0.05$) in the streptozocin group was minded when compared with both; the 5 mg/kg (fig. 3-18) and the 10 mg/kg (3-19) of the atorvastatin-streptozocin treated groups. At the same time, a significant decrease ($p<0.05$) in the same streptozocin group was seen when confronted with the 20 mg/kg atorvastatin-streptozocin treated group (fig. 3-20). All the 5 mg/kg, 10 mg/kg and 20 mg/kg atorvastatin-streptozocin treated groups showed a non-significant change ($p>0.05$) when set side by side with each other. The figure (3-16) indicates the results given above on glutathione reductase.

Table (3-10): descriptive statistics for the score of GR expression in groups treated with streptozocin and atorvastatin.

groups	Sample No.	25% Percentile (Q1)	75% Percentile (Q3)	Minimum value	Maximum value	Mean± SD	Median (Q2)
Control	6	3.750	4.000	3.000	4.000	3.833± 0.4082	4.000
Streptozocin	6	1.000	2.000	1.000	2.000	1.667± 0.5164	2.000
Streptozocin.+ Atorvastatin.5mg	6	3.000	4.000	3.000	4.000	3.333± 0.5164	3.000
Streptozocin.+ Atorvastatin.10mg	6	3.000	4.000	3.000	4.000	3.500± 0.5477	3.500
Streptozocin.+ Atorvastatin.20mg	6	3.750	4.000	3.000	4.000	3.833± 0.4082	4.000

*Q: quartile.

Table (3-11): Kruskal-Wallis test for the score of GR expression in groups treated with streptozocin and atorvastatin.

Test	Statistical value	P value
Kruskal-Wallis statistic	18.94	0.0008

Table (3-12): Effects of multiple doses of atorvastatin combined with streptozocin and streptozocin alone on glutathione reductase.

Dunn's Multiple Comparison Test	Difference in rank sum	Significancy
Control vsStreptozocin.	17.50	S
Control vs Streptozocin.+Atorvastatin.5	6.000	NS
Control vs Streptozocin.+Atorvastatin.10	4.000	NS
Control vs Streptozocin.+Atorvastatin.20	0.0000	NS
Streptozocin. vs Streptozocin.+Atorvastatin.5	-11.50	S
Streptozocin. vs Streptozocin.+Atorvastatin.10	-13.50	S
Streptozocin. vsStreptozocin.+Atorvastatin.20	-17.50	S
Streptozocin.+Atorvastatin.5 vs Streptozocin.+Atorvastatin.10	-2.000	NS
Streptozocin.+Atorvastatin.5 vs Streptozocin.+Atorvastatin.20	-6.000	NS
Streptozocin.+Atorvastatin.10 vs Streptozocin.+Atorvastatin.20	-4.000	NS

S: Significant difference ($p<0.05$).

NS: non-significant ($p>0.05$).

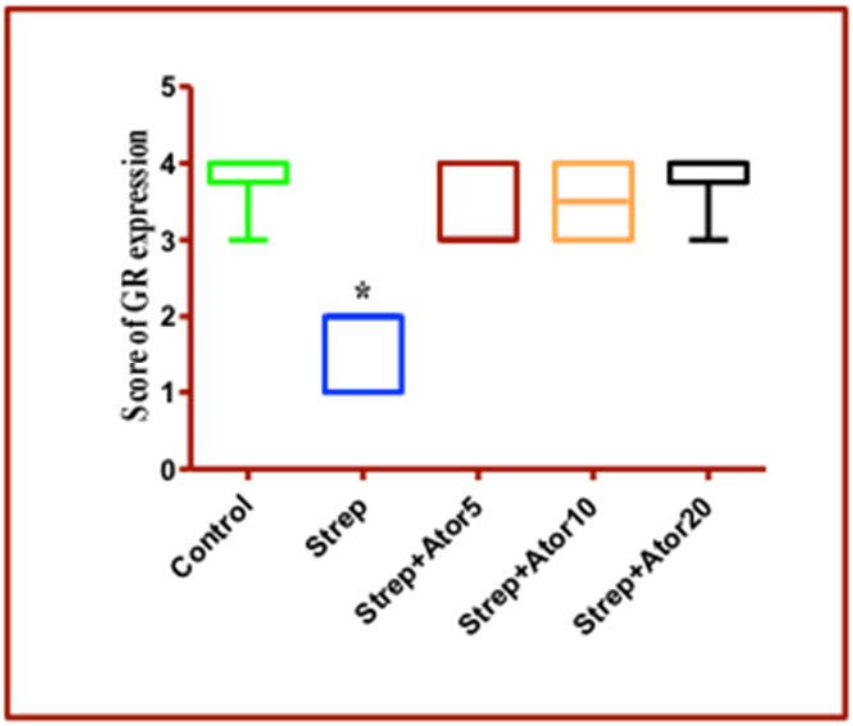

Figure (3-16): box and whisker plot showing the effects of multiple doses of atorvastatin combined with streptozocin and streptozocin alone on the expression of glutathione reductase marker.

***: significantly different compared with every other groups.**

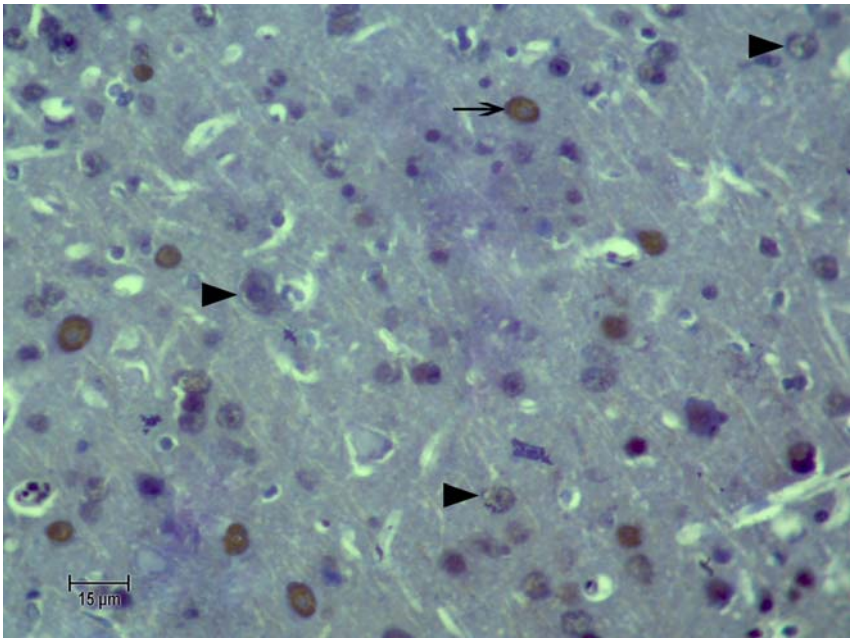

Figure (3-17): Immunohistochemical staining for hippocampus expressions of GR marker in the group administered 3 mg/kg streptozocin. (Arrows represent positively stained cells while heads of arrows represent the negative one**).**

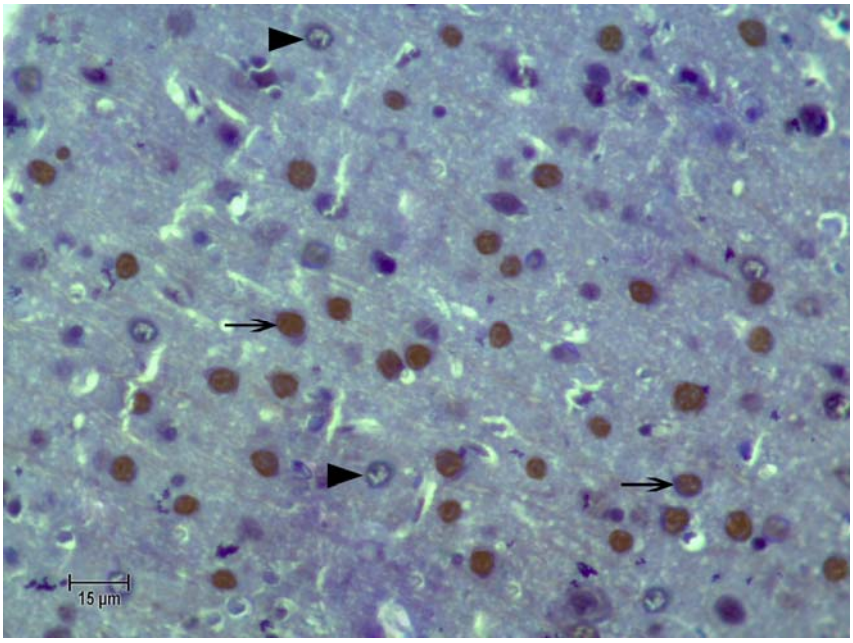

Figure (3-18): Immunohistochemical staining for hippocampus expressions of GR marker in the group administered streptozocin +5 mg/kg atorvastatin. (Arrows represent positively stained cells while heads of arrows represent the negative one**).**

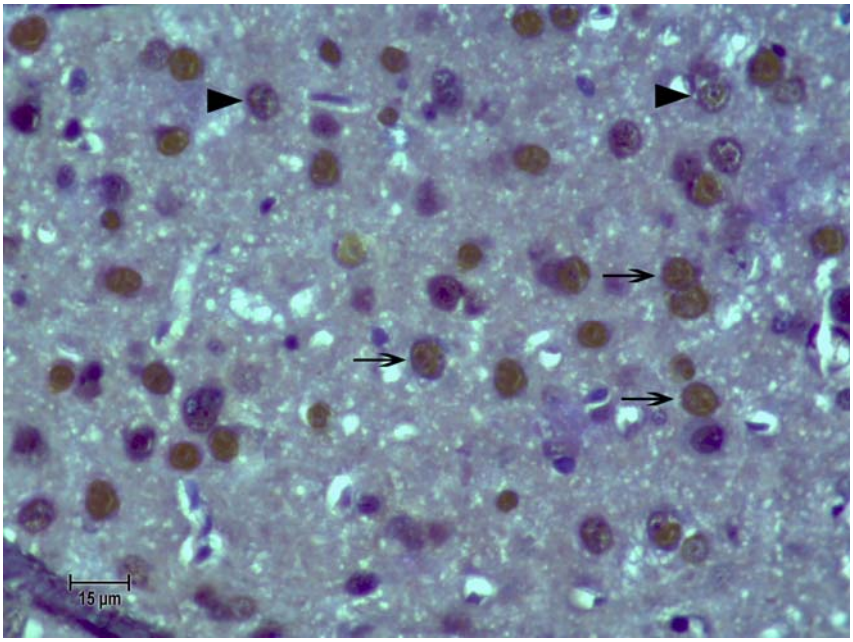

Figure (3-19): Immunohistochemical staining for hippocampus expressions of GR marker in the group administered streptozocin +10mg/kg atorvastatin. (Arrows represent positively stained cells while heads of arrows represent the negative one**).**

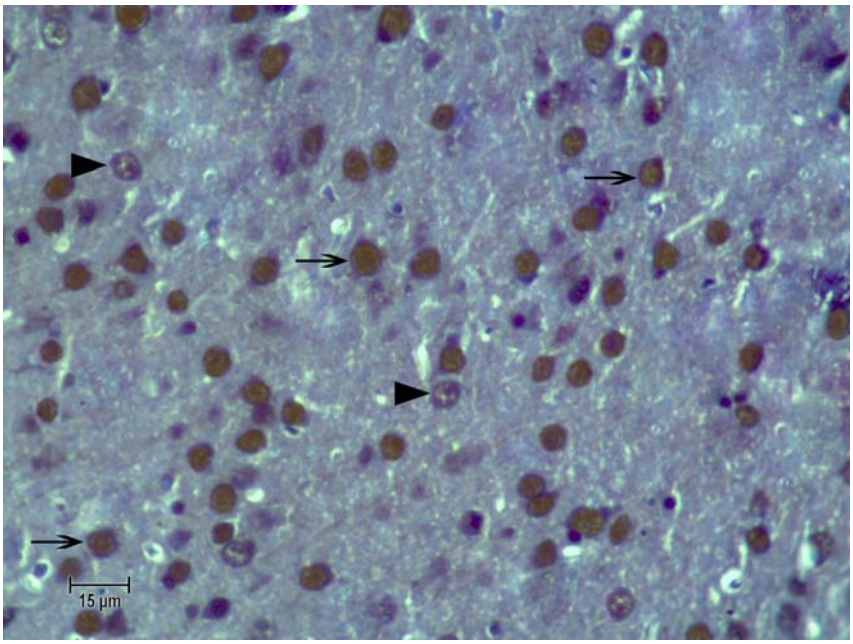

Figure (3-20): Immunohistochemical staining for hippocampus expressions of GR markers in the group administered streptozocin + 20mg/kg atorvastatin. (Arrows represent positively stained cells while heads of arrows represent the negative one**).**

3.5. Effects of various doses of atorvastatin on neuronal Nitric Oxide Synthase (nNOS):

The descriptive statistics, which represent the mean, median, minimum value and maximum value for the score of nNOS expression in groups treated with atorvastatin orally, are summarized in table (3-13). Analysis of data statistically by Kruskal-Wallis test showed a significant difference ($p<0.05$) among the groups (table 3-14). Dunn's Multiple Comparison Test was done and the table (3-15) shows that there is a non significant decrease ($p>0.05$) in the score of the nNOS expression of the group of rats treated with 5 mg/kg of atorvastatin (fig. 3-23) orally when compared with the control (fig. 3-22). Moreover, there is another non-significant decrease ($p>0.05$) in the group of rats administered 10 mg/kg of atorvastatin (fig. 3-24) when confronted with the control group. On the contrary, table (3-15) demonstrated a significant decrease ($p<0.05$) in the 20 mg/kg atorvastatin treated group (fig. 3-25) when compared with the control. At the same time, all the 5 mg/kg, 10 mg/kg and 20 mg/kg atorvastatin treated groups showed a non-significant change ($p>0.05$) when set side by side each other. The figure (3-21) exhibits the effects of different doses of atorvastatin on neuronal nitric oxide synthase.

Table (3-13): descriptive statistics for the score of nNOS expression in groups treated with atorvastatin.

groups	Sample No.	25% Percentile (Q1)	75% Percentile (Q3)	Minimum value	Maximum value	Mean± SD	Median (Q2)
Control	6	2.000	2.250	2.000	3.000	2.167± 0.4082	2.000
Atorvastatin 5mg	6	1.750	2.000	1.000	2.000	1.833± 0.4082	2.000
Atorvastatin 10mg	6	1.000	2.000	1.000	2.000	1.667± 0.5164	2.000
Atorvastatin 20mg	6	1.000	1.250	1.000	2.000	1.167± 0.4082	1.000

*Q: quartile.

Table (3-14): Kruskal-Wallis test for the score of nNOS expression in groups treated with atorvastatin.

Test	Statistical value	P value
Kruskal-Wallis statistic	10.50	0.0148

Table (3-15): Effects of various doses of atorvastatin on neuronal Nitric Oxide Synthase (nNOS)

Dunn's Multiple Comparison Test	**Difference in rank sum**	**Significancy**
Control vs Atorvastatin.5mg	3.250	NS
Control vs Atorvastatin.10mg	5.167	NS
Control vs Atorvastatin.20mg	10.92	S
Atorvastatin.5 vs Atorvastatin.10mg	1.917	NS
Atorvastatin.5 vs Atorvastatin.20mg	7.667	NS
Atorvastatin.10 vs Atorvastatin.20mg	5.750	NS

S: Significant difference ($p<0.05$).

NS: non-significant ($p>0.05$).

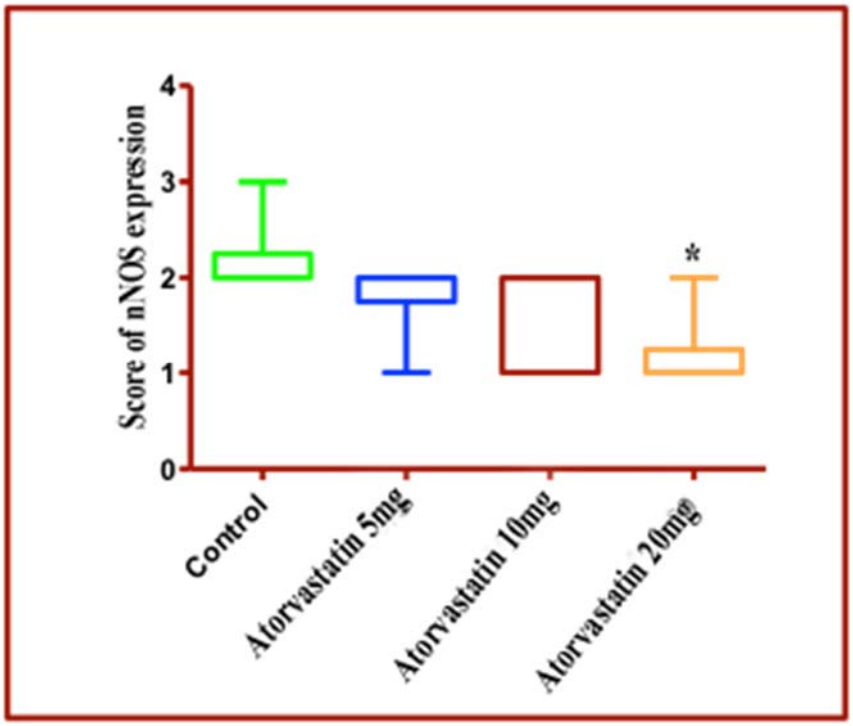

Figure (3-21): box and whisker plot showing the effects of different doses of atorvastatin on the expression of the neuronal nitric oxide synthase marker.

*: **significantly different compared with the control.**

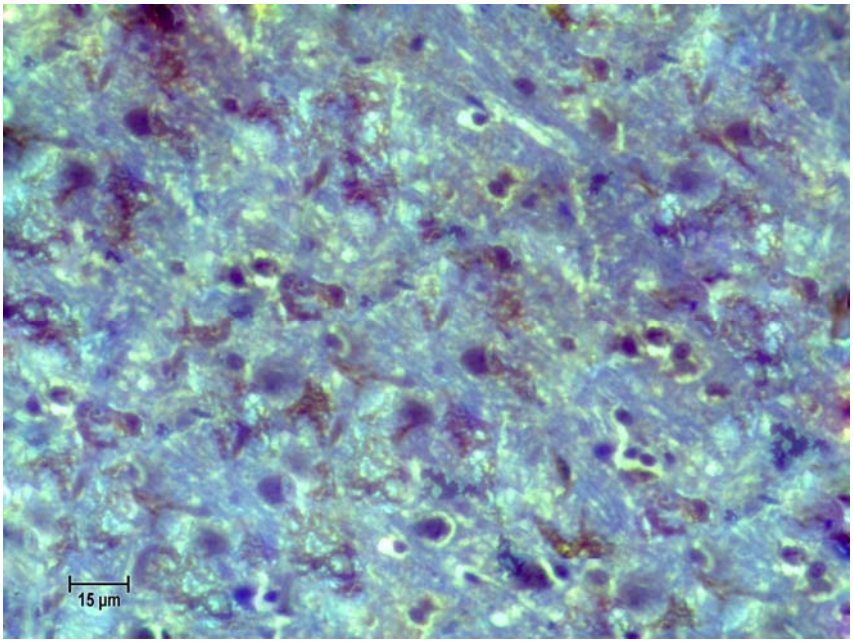

Figure (3-22): Immunohistochemical staining for hippocampus expressions of nNOS marker in the control. (Scale bar at left lower corner represents 15 µm (400 ×)).

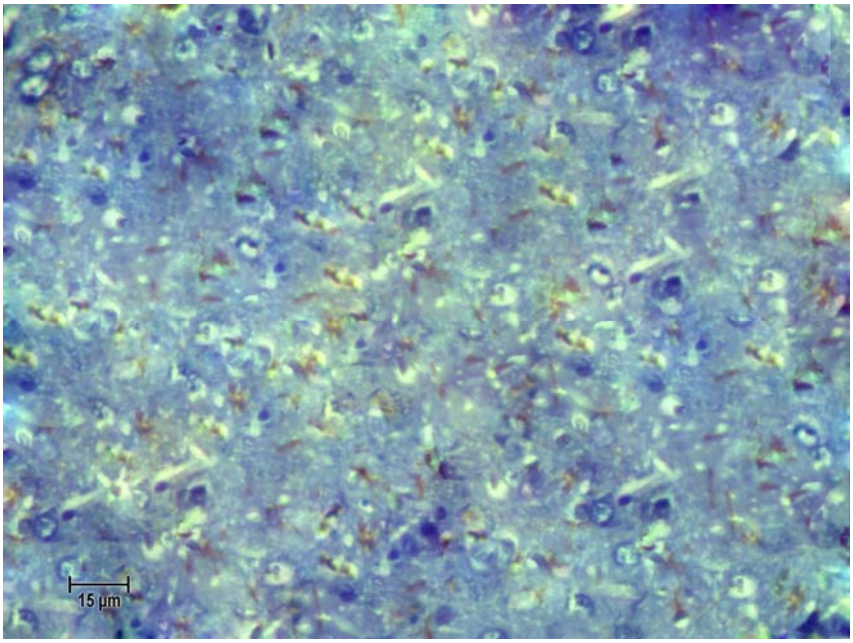

Figure (3-23): Immunohistochemical staining for hippocampus expressions of nNOS marker in the group treated with 5 mg/kg atorvastatin.

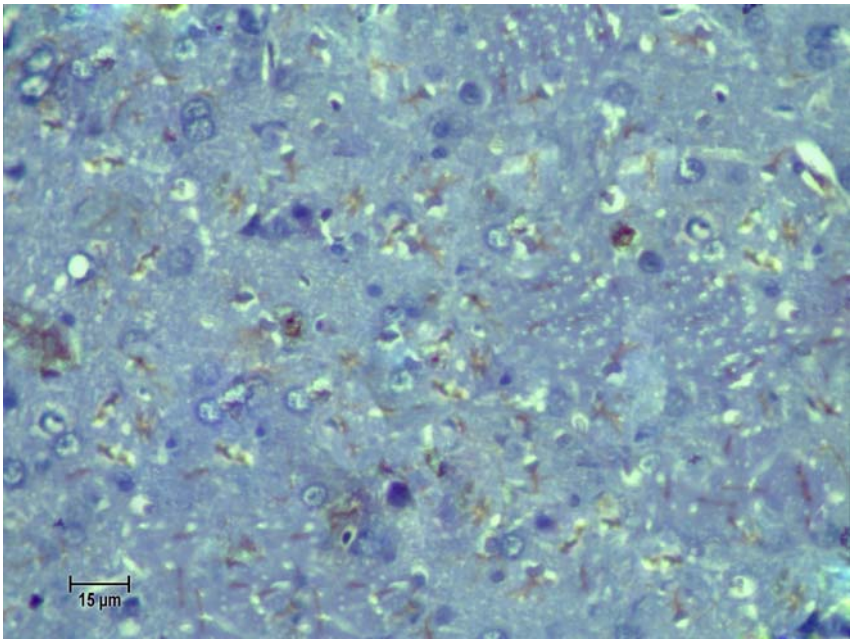

Figure (3-24): Immunohistochemical staining for hippocampus expressions of nNOS marker in the group treated with 10 mg/kg atorvastatin.

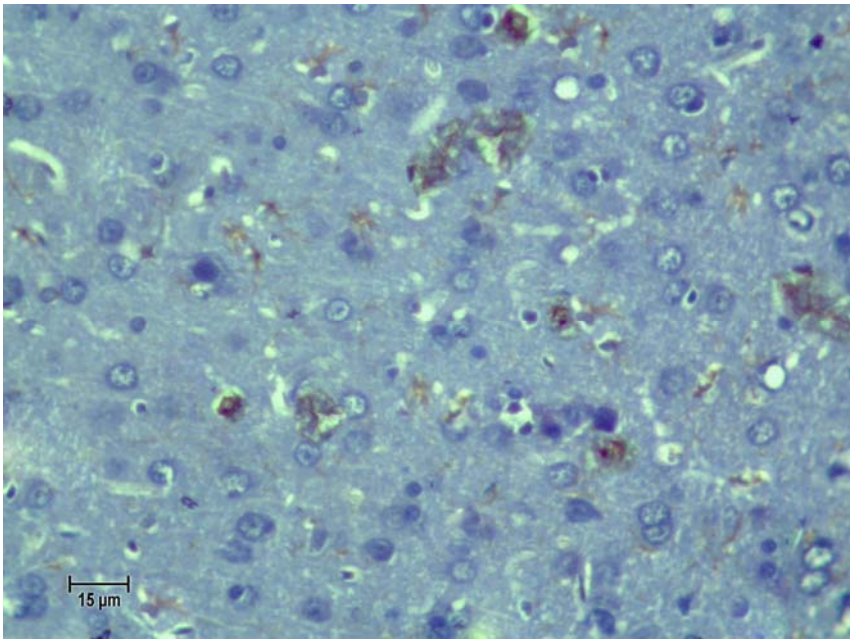

Figure (3-25): Immunohistochemical staining for hippocampus expressions of nNOS marker in the group treated with 20 mg/kg atorvastatin.

3.6. Effects of streptozocin alone and different doses of atorvastatin combined with streptozocin on neuronal Nitric Oxide Synthase (nNOS):

The descriptive statistics, which represent the mean, median, minimum value and maximum value for the score of nNOS expression in the streptozocin group and groups treated with atorvastatin orally after administered streptozocin intrathecally, are recapitulated in table (3-16). Analysis of data statistically by Kruskal-Wallis test revealed a significant difference ($p<0.05$) among the groups (table 3-17). Dunn's Multiple Comparison Test was done and the table (3-18) assures that there is a significant increase ($p<0.05$) in the score of the nNOS expression of the group administered 3 mg/kg of streptozocin intrathecally (fig. 3-27) when compared with the control. On the contrary, there was no significant change ($p>0.05$) when the control was compared against the groups treated with 3 mg/kg of streptozocin intrathecally combined with 5 mg/kg, 10 mg/kg and 20 mg/kg of atorvastatin orally. Table (3-18) demonstrated that a non-significant decrease ($p>0.05$) in the 5 mg/kg atorvastatin-streptozocin treated group (fig. 3-28) was minded when compared with the streptozocin group. Meanwhile, there is a significant decrease ($p<0.05$) in the 10 mg/kg atorvastatin-streptozocin treated group (fig. 3-29) when confronted with the streptozocin group. At the same time, significant decrease ($p<0.05$) in the 20 mg/kg atorvastatin-streptozocin treated group (fig. 3-30) was observed when compared with the streptozocin group. All the 5 mg/kg, 10 mg/kg and 20 mg/kg atorvastatin-streptozocin treated groups showed a non significant change ($p>0.05$) when compared with each other. The figure (3-26) indicates the results given above on neuronal Nitric Oxide Synthase.

Table (3-16): descriptive statistics for the score of nNOS expression in groups treated with streptozocin and atorvastatin.

groups	Sample No.	25% Percentile (Q1)	75% Percentile (Q3)	Minimum value	Maximum value	Mean± SD	Median (Q2)
Control	6	2.000	2.250	2.000	3.000	2.167±0.4082	2.000
Streptozocin.	6	3.000	3.250	3.000	4.000	3.167±0.4082	3.000
Streptozocin.+ Atorvastatin.5mg	6	2.000	3.000	2.000	3.000	2.333±0.5164	2.000
Streptozocin.+ Atorvastatin.10mg	6	2.000	2.250	2.000	3.000	2.167±0.4082	2.000
Streptozocin.+ Atorvastatin.20mg	6	1.750	2.000	1.000	2.000	1.833±0.4082	2.000

*Q: quartile.

Table (3-17): Kruskal-Wallis test for the score of nNOS expression in groups treated with streptozocin and atorvastatin.

Test	Statistical value	P value
Kruskal-Wallis statistic	16.59	0.0023

Table (3-18): Effects of streptozocin alone and different doses of atorvastatin combined with streptozocin on neuronal Nitric Oxide Synthase (nNOS)

Dunn's Multiple Comparison Test	Difference in rank sum	Significancy
Control vsStreptozocin.	-12.50	S
Control vs Streptozocin.+Atorvastatin.5	-2.333	NS
Control vs Streptozocin.+Atorvastatin.10	0.0000	NS
Control vs Strp.+Atorvastatin.20	4.000	NS
Streptozocin. vs Streptozocin.+Atorvastatin.5	10.17	NS
Streptozocin. vs Streptozocin.+Atorvastatin.10	12.50	S
Streptozocin. vs Strp.+Atorvastatin.20	16.50	S
Streptozocin.+Atorvastatin.5 vs Streptozocin.+Atorvastatin.10	2.333	NS
Streptozocin.+Atorvastatin.5 vs Strp.+Atorvastatin.20	6.333	NS
Streptozocin.+Atorvastatin.10 vs Strp.+Atorvastatin.20	4.000	NS

S: Significant difference ($p<0.05$).

NS: non-significant ($p>0.05$).

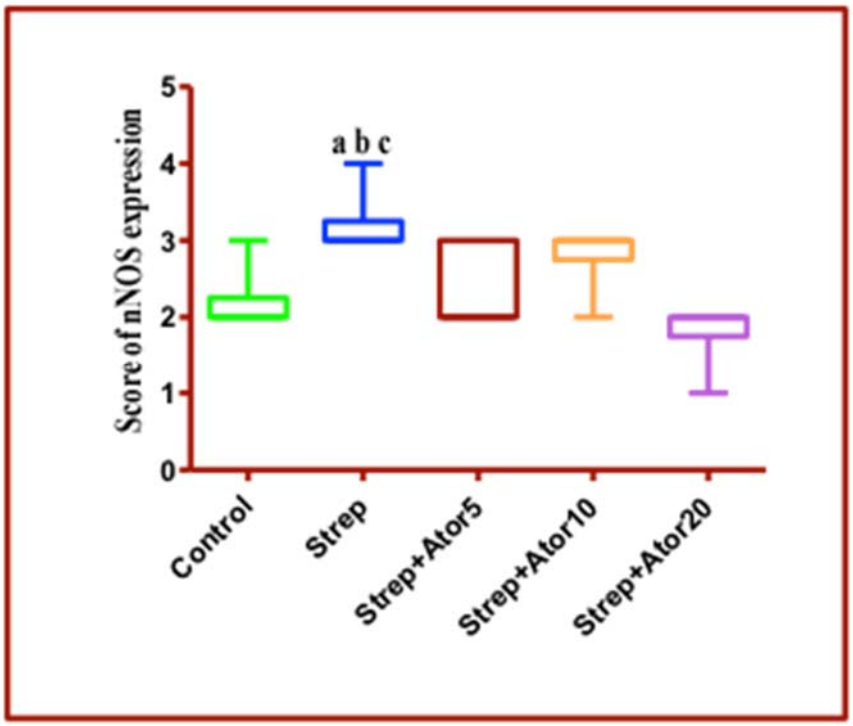

Figure (3-26): box and whisker plot showing the effects of streptozocin alone and different doses of atorvastatin combined with streptozocin on the expressionof the neuronal nitric oxide synthase marker.

[a]: significantly different compared with the control.

[b]: significantly different compared with Streptozocin-Atorvastatin 10mg/kg group.

[c]: significantly different compared with Streptozocin-Atorvastatin 20mg/kg group.

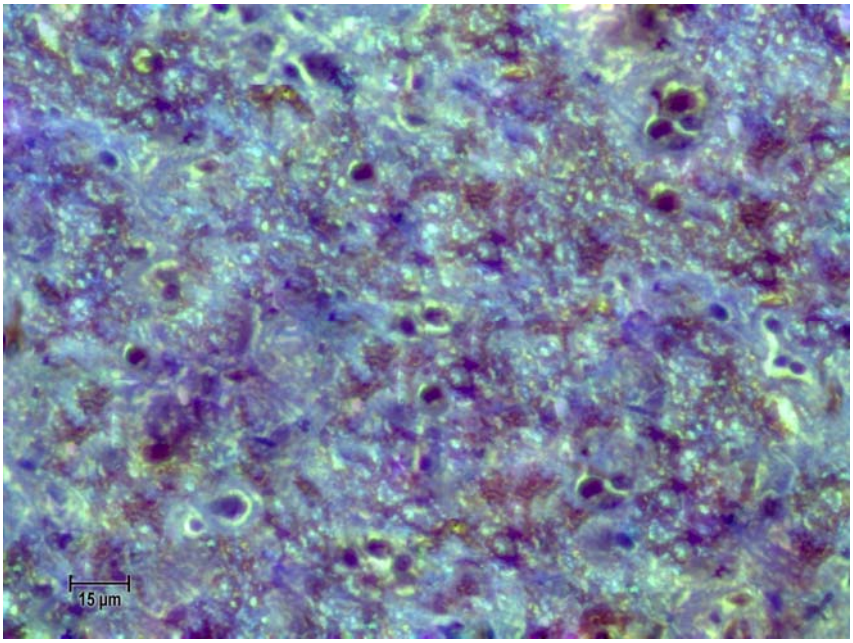

Figure (3-27): Immunohistochemical staining for hippocampus expressions of nNOS marker in the group administered 3 mg/kg streptozocin. (Scale bar at left lower corner represents 15 µm (400 ×)).

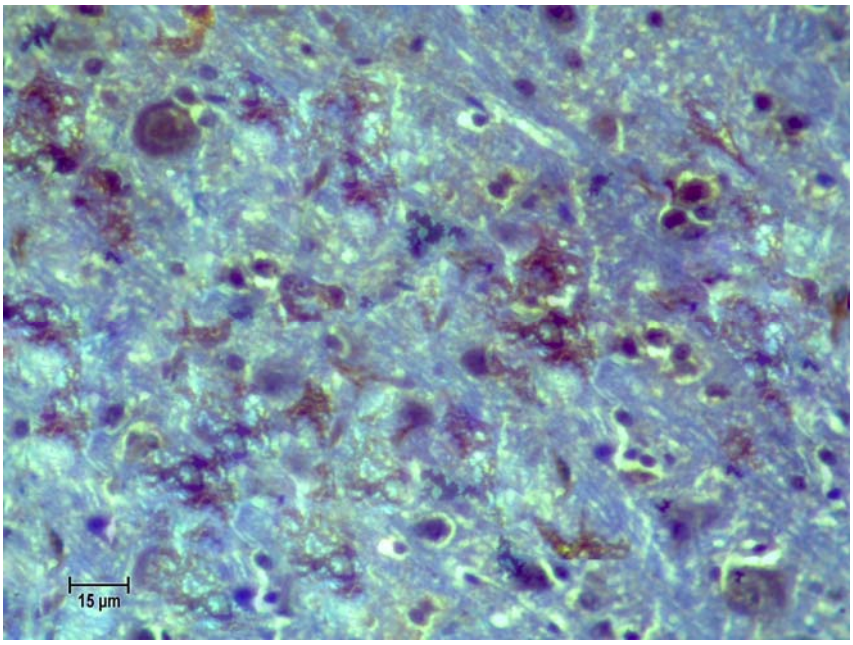

Figure (3-28): Immunohistochemical staining for hippocampus expressions of nNOS marker in the group treated with 5 mg/kg atorvastatin + streptozocin.

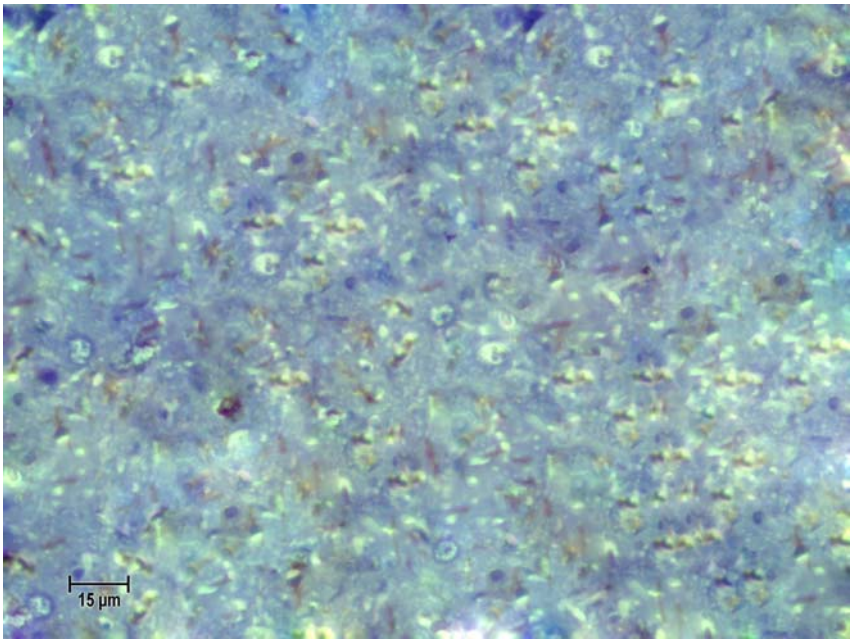

Figure (3-29): Immunohistochemical staining for hippocampus expressions of nNOS marker in the group administered streptozocin + 10 mg/kg atorvastatin.

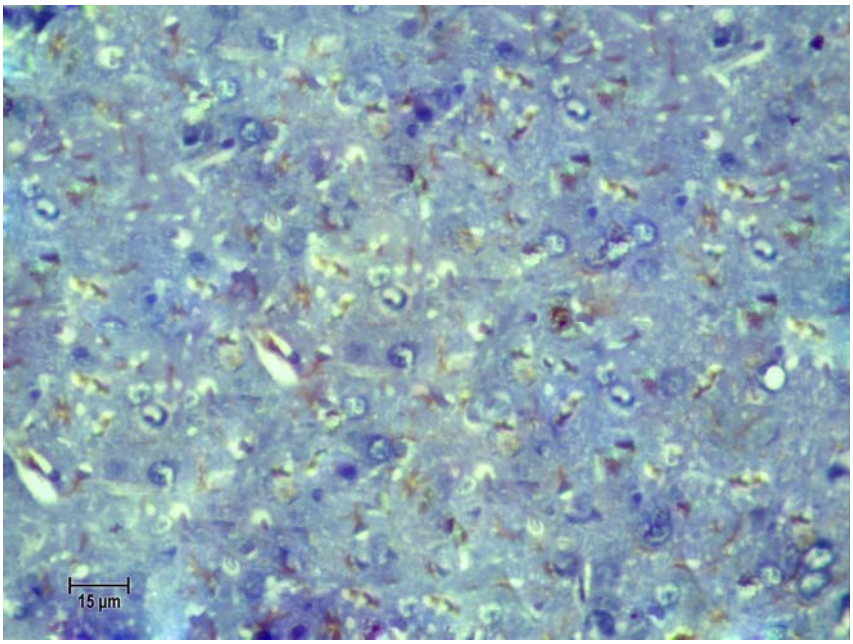

Figure (3-30): Immunohistochemical staining for hippocampus expressions of nNOS marker in the group treated with 20 mg/kg atorvastatin + streptozocin.

3.7. Effects of different doses of atorvastatin, streptozocin alone and various atorvastatin-streptozocin doses on lipid profile:

3.7.1. Serum cholesterol level

Table (3-19) demonstrates that as the atorvastatin doses increased from 5 mg/kg to 10 mg/kg and to 20 mg/kg, a more significant reduction in serum cholesterol level ($p<0.05$) was seen when compared with the control values. Meanwhile, there is a non-significant changes in serum cholesterol ($p>0.05$) in rats administered intrathecal streptozocin alone compared with control.

The groups that treated with 5 mg/kg,10 mg/kg and 20 mg/kg of atorvastatin orally after administered intrathecal streptozocin injection showed a significant reduction in serum cholesterol level ($p<0.05$) when compared with the control and the group administered streptozocin alone. Figure (3-31) shows the effects of atorvastatin and streptozocin on serum cholesterol level.

3.7.2. Serum triglyceride level

Table (3-19) displays that as the atorvastatin doses increased from 5 mg/kg to 10 mg/kg and to 20 mg/kg, a more significant reduction in serum triglyceride level ($p<0.05$) was seen when contrasted with the control values. In the meantime, there is a non-significant changes in serum triglyceride ($p>0.05$) in rats administered streptozocin alone when compared with the control. On the other hand, the groups treated with 5 mg/kg, 10 mg/kg and 20 mg/kg of atorvastatin orally combined with intrathecal streptozocin injection showed a significant reduction in serum triglyceride level ($p<0.05$) when compared with the group administered streptozocin alone and with control values. Figure (3-32) demonstrates the effects of different doses of atorvastatin, streptozocin alone and various atorvastatin-streptozocin doses on Serum triglyceride level.

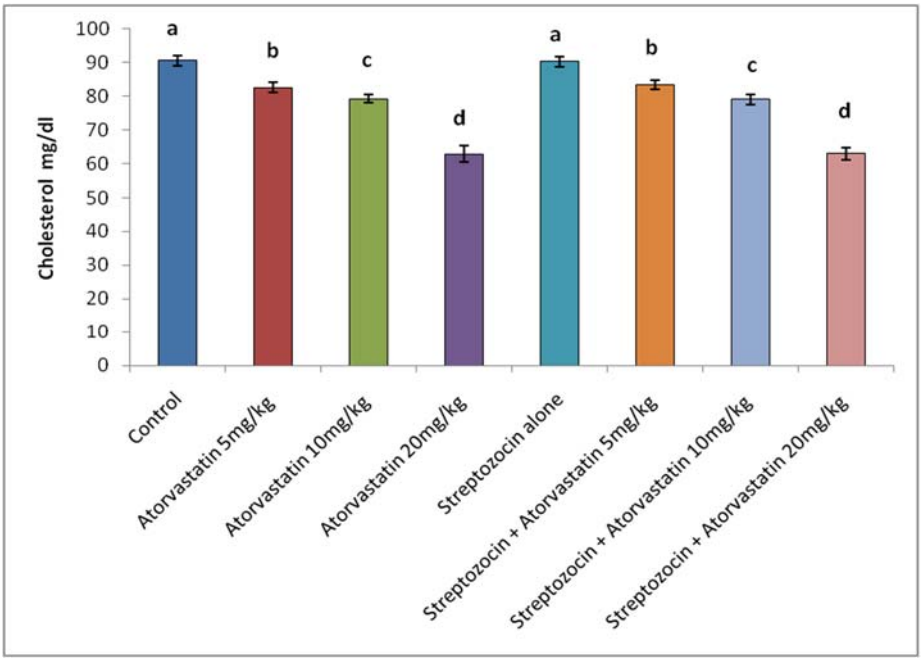

Figure (3-31): Effects of different doses of atorvastatin, streptozocin alone and various atorvastatin-streptozocin doses on serum cholesterol level.

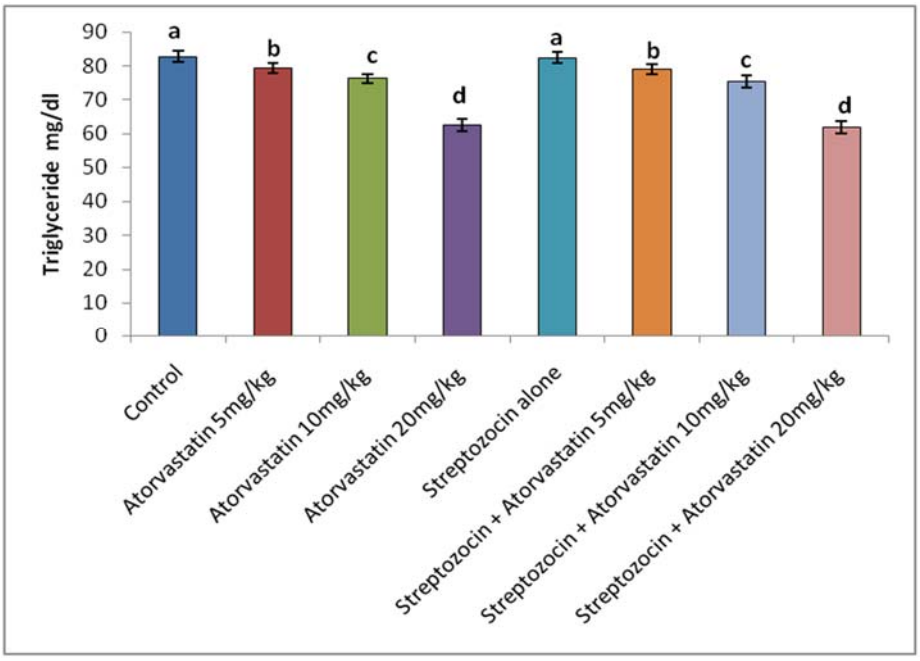

Figure (3-32): Effects of different doses of atorvastatin, streptozocin alone and various atorvastatin-streptozocin doses on serum triglyceride level.

3.7.3. Serum Low Density Lipoprotein (LDL) level.

Table (3-19) demonstrates that as the atorvastatin doses increased from 5 mg/kg to 10 mg/kg and to 20 mg/kg, a higher significant reduction in serum LDL level ($p<0.05$) was noticed when compared with the control group. On the contrary, there is a non-significant changes in serum LDL ($p>0.05$) in rats administered streptozocin alone when contrasted with the control values. Moreover, the groups treated with 5 mg/kg, 10 mg/kg and 20 mg/kg of atorvastatin orally with intrathecal streptozocin injection showed a significant reduction in serum LDL level ($p<0.05$) when compared with the group administered streptozocin alone and with control one. As these doses of atorvastatin increased, the level of serum LDL decreased. Figure (3-33) makes evident the effects of different doses of atorvastatin with and without streptozocin on Serum LDL level.

3.7.4. Serum High Density Lipoprotein (HDL) level

Table (3-19) indicates that there is a non-significant changes ($p>0.05$) observed in the serum HDL level in the all groups of this study that administered different doses of atorvastatin orally with or without intrathecal streptozocin injection when compared with the control values. Figure (3-34) shows the effects of atorvastatin and streptozocin on serum HDL level.

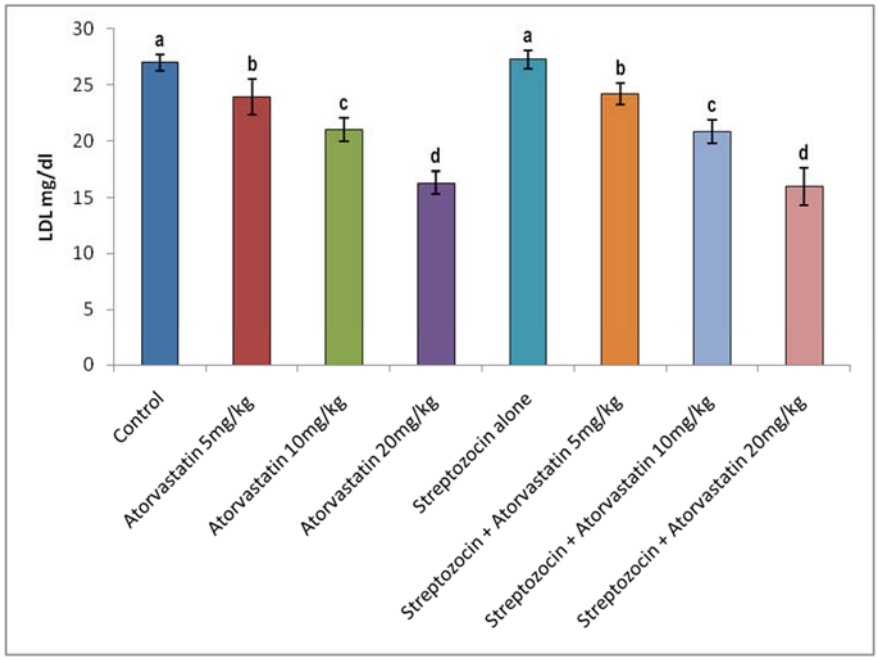

Figure (3-33): Effects of different doses of atorvastatin, streptozocin alone and various atorvastatin-streptozocin doses on serum LDL level.

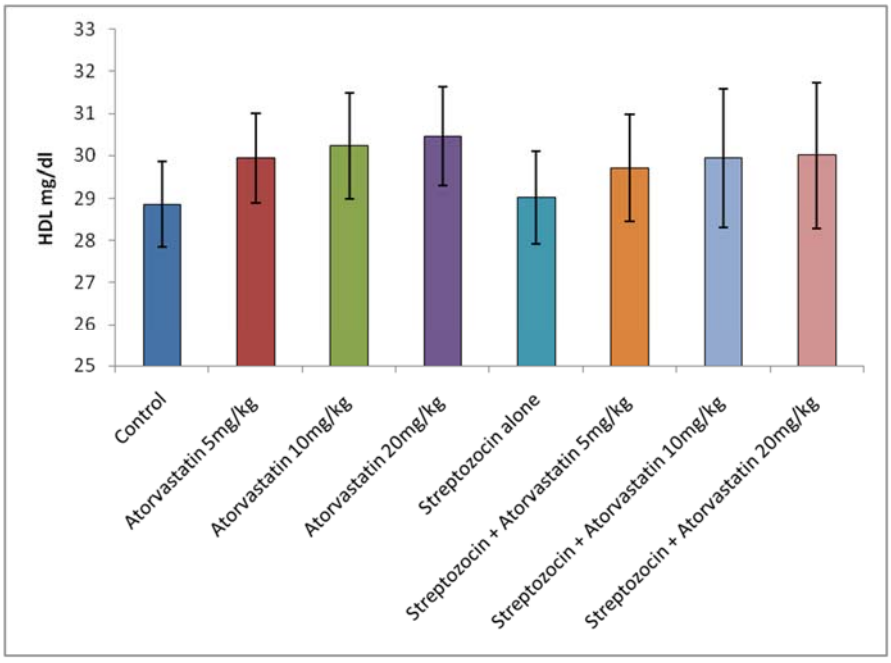

Figure (3-34): Effects of different doses of atorvastatin, streptozocin alone and various atorvastatin-streptozocin doses on serum HDL level.

Table (3-19): Effects of different doses of atorvastatin, streptozocin alone and various atorvastatin-streptozocin doses on lipid profile.

Groups	Mean ± SD Level			
	Cholesterol mg/dl	Triglyceride mg/dl	LDL mg/dl	HDL mg/dl
Control	90.51±1.43$^{(a)}$	82.73± 1.70$^{(a)}$	26.98±0.70$^{(a)}$	28.85±1.00
Atorvastatin. 5mg	82.53±1.50$^{(b)}$	79.18±1.53$^{(b)}$	23.91±1.59$^{(b)}$	29.95±1.05
Atorvastatin. 10mg	79.20±1.28$^{(c)}$	76.21±1.32$^{(c)}$	21.01±1.24$^{(c)}$	30.23±1.25
Atorvastatin. 20mg	62.81±2.35$^{(d)}$	62.31±1.79$^{(d)}$	16.30±0.98$^{(d)}$	30.46±1.17
Streptozocin	90.26±1.53$^{(a)}$	82.43±1.53$^{(a)}$	27.25±0.84$^{(a)}$	29.01±1.09
Streptozocin.+ Atorvastatin.5 mg	83.48±1.35$^{(b)}$	78.98±1.45$^{(b)}$	24.20±0.98$^{(b)}$	29.71±1.27
Streptozocin.+ Atorvastatin.10 mg	78.95±1.45$^{(c)}$	75.28±1.80$^{(c)}$	20.81±1.04$^{(c)}$	29.95±1.64
Streptozocin.+ Atorvastatin.20 mg	62.93±1.87$^{(d)}$	61.86±1.83$^{(d)}$	15.95±1.60$^{(d)}$	30.01±1.73

-Means with different letters differ significantly ($P < 0.05$).

-Means with the same letters are not differ significantly ($P > 0.05$).

-Cholesterol: for conversion from mg/dl to mmol/l multiply by 0.0259

-TG: for conversion from mg/dl to mmol/l multiply by 0.0113

-LDL: for conversion from mg/dl to mmol/l multiply by 0.0259

-HDL: for conversion from mg/dl to mmol/l multiply by 0.0259

Chapter Four

Discussion

4.1. Effects of different doses of Atorvastatin on Glial Fibrillary Acidic Protein (GFAP)

Normal brain aging is characterized by many macroscopic changes including decreased brain volume and weight, and microscopic alterations such as changes in blood–brain barrier permeability, modifications in the extracellular compartment, and both cellular and biochemical alterations in glia and neuronal cells [(164)] . Also, proliferation of astrocytes with the morphological changes associated with activation has been documented with age, which in turn leads to an increase in the expression of GFAP in the brain [(165)]. Hayakawa *et al*. 2007 showed that increase GFAP immunoreactivity was observed in hippocampus of mice older than 50 weeks [(166)].

The results collected in the present study shows a non significant change in the GFAP expression in the hippocampus when a dose of 5 mg/kg or 10 mg/kg of atorvastatin is given orally. These findings come in agreement with the study of Piermartiri T.C.B *et al.* in which they showed that there was no significant alteration in GFAP when 10 mg/ kg of atorvastatin treatment was administered [(117)].

Meanwhile, this study shows that there is a significant reduction in the GFAP expression when a dose of 20 mg/kg atorvastatin is administered in comparison with the control and 5 mg/kg atorvastatin groups. The possible explanation may be because the GFAP as an indicator for reactive astrocytosis, which is a source of cytokines including IL-1β, TNF-α, and IL-6[(167)]. Reactive astrocytosis may exacerbate inflammation by inducing the migration of other leukocytes into the brain, interrupting blood-brain-barrier function [(168,169)] and producing reactive oxygen species [(170,171)]. The results showed that 20 mg/kg atorvastatin treatment reduced GFAP-positive astrocytes in the hippocampus. This atorvastatin-mediated suppression of

astrocytosis may contribute to inhibition of neuroinflammation and neuronal loss, thus exert a neuroprotective effect.

4.2. Effects of Streptozocin alone and various doses of Atorvastatin combined with Streptozocin on Glial Fibrillary Acidic Protein (GFAP)

After cellular injury, the GFAP is synthesized plentifully, thus, it represents itself more conspicuously within the reactive gliosis process [172]. Generalized astrogliosis, demonstrated by cellular hypertrophy and by an increase in expression of GFAP and astroglial S100B protein, was routinely observed in postmortem tissues from AD patients [173, 174, 175, 176].

In the AD human tissue, the main astroglial reaction found is represented by prominent astrogliosis, mostly observed in the cells surrounding amyloid plaques [177]. Importantly, activated astrocytes are capable of accumulating large amounts of Aβ; the later being taken up by astrocytes in association with neuronal debris. Beside, reactive astrocytes seem to accumulate large amounts of neuronal subtype of nicotinic cholino-receptor (α-7nAChRs), which is known to have an exceptionally high affinity to β-amyloid. Astroglial β-amyloid deposits are clearly associated with plaques; processes of activated astrocytes were also reported to participate in plaques formation [177].

The present findings indicate that there is a significant increase in the expression of GFAP in the group of rats administered 3 mg/kg of streptozocin intrathecally and this come in agreement with previous study [138].

Although there are few studies regarding GFAP hippocampal alterations in STZ-treated rodents, Shoham S. *et al.* 2007 investigated that

STZ increased the number of activated astrocytes in the CA1 and other regions of the hippocampus. Thus increasing in GFAP expression was seen after ICV STZ [138].

In this study, the significant reduction in the GFAP expression was occured in the rats administered 20 mg/kg atorvastatin orally after brain damage from 3 mg/kg intrathecal injection of streptozocin and this in agreement with the previous study indicated that the treatment with atorvastatin 30 mg/kg reduced GFAP staining density compared with the amyloid precursor protein transgenic mice group [178].

The mechanism that the streptozocin causes an upregulation of GFAP may be related to the astrocytes. Astrocytes in the healthy brain usually do not express iNOS, but after ischemic, traumatic, neurotoxic, or inflammatory damage, activation of NF-Kappa-B plays an important role in the expression of many proinflammatory molecules in astrocytes [the expression of iNOS and proinflammatory cytokines (TNF- α, IL-1β, and IL-6)] [165]. The expression of iNOS in the astrocytes causes production of excessive amount of NO which in turn led to upregulation of GFAP expression in reactive astrocytes by using the guanylate cyclase (GC)–cGMP-activated protein kinase (PK-G) signaling pathway to induce this expression [179].

Statins exert anti-inflammatory effects by reducing the production of pro-inflammatory molecules such as tumor necrosis factor-α (TNF-α) and inducible nitric oxide synthase (iNOS) in astrocytes [180] and that cause a decrease in GFAP expression indicating reduce in astrocytes activation that in turn cause a reduction in neuroinflammation response. This study shows that the effect of atorvastatin on expression of GFAP is happened on a high dose (20 mg/kg) that means the effect is dose-dependent.

4.3. Effects of different doses of Atorvastatin on Glutathione Reductase (GR)

Glutathione (γ-glutamylcysteinylglycine) is the most abundant thiol in cells [181, 182]. Glutathione protects cells against exogenous and endogenous toxins, including reactive oxygen species (ROS) and reactive nitrogen species (RNS). Such radical species are removed via non-enzymatic reduction with GSH, whereas the removal of hydroperoxides requires enzymatic catalysis by glutathione peroxidase [183]. Both reactions lead to the generation of glutathione disulfide (GSSG, or oxidized glutathione), which is reduced back to GSH by glutathione reductase that uses NADPH from the pentose phosphate shunt [183].

The results of the this study shows that there is a significant increase in the expression of GR in the hippocampus of rats administered 20 mg/kg atorvastatin orally compared with control, 5 mg/kg and 10 mg/kg atorvastatin groups in agreement with previous study [184]. Barone E. *et al*. (2011) found that high doses of atorvastatin treatment reduced lipoperoxidation, protein oxidation and nitration, and increased GSH levels in parietal cortex of aged beagles [185].

The most likely explanation for the increase of GSH by atorvastatin was attributed to the antioxidant effect of atorvastatin that results from inhibition of mevalonate pathway. This effect leads to a reduction in the synthesis of important intermediates including isoprenoids (geranylgeranyl pyrophosphate and farnesyl pyrophosphate). These isoprenoids serve as lipid attachments for intracellular signaling molecules (Rho, Rac, Ras and G proteins) which depend on isoprenylation for function and membrane localization [186].

In general Rho causes a decrease in eNOS while, Rac leads to increase in NAD(P)H oxidase which cause increase production of ROS [187, 188]. Atorvastatin causes reduction in the synthesis of Rac and Rho, which in turn lead to inhibition of NAD(P)H oxidase activity and upregulation of eNOS, respectively, that attenuate endothelial reactive oxygen species (ROS) formation [186].

4.4. Effects of multiple doses of Atorvastatin combined with Streptozocin and Streptozocin alone on Glutathione Reductase (GR)

Under physiological conditions, a balance between pro-oxidant and anti-oxidant stimuli cell is regulated; while, certain stressors, damage, or diseases may affect this equilibrium and increase production of reactive nitrogen species (RNS) and reactive oxygen species (ROS), which in turn may react with endogenous molecules including proteins, lipids, carbohydrates, DNA and RNA [189] leading to cellular dysfunction resulting from their oxidative damage. Several lines of evidence have shown that the brains of individuals with Alzheimer's disease demonstrated elevation in the oxidative stress levels [190,191].

The present work indicates that the expression of glutathione reductase is significantly reduced in the group of rats administered 3 mg/kg streptozocin intrathecally compared with control group. These findings are in accordance with the results of previous studies [192, 193].

Ishrat T. *et al.* (2009) observed a significant decrease in reduced glutathione (GSH) and antioxidant enzymes (glutathione peroxidase GPx and glutathione reductase GR) in the hippocampus and cerebral cortex of ICV-STZ treated rats [141]. the results of this study were in agreement with the study conducted by Javed H. *et al*. (2013) indicated that the activity of

antioxidant enzymes (glutathione peroxidase, glutathione reductase, glutathione-S-transferase, catalase, and superoxide dismutase) was decreased in rats treated with 3 mg/kg STZ intracerebroventricular as compared with control group [194].

Tota S. *et al.* (2011) also showed that STZ caused oxidative stress as evidenced by significant decrease in GSH level. In addition, there was a significant rise in reactive oxygen species (ROS) and nitrite levels in brain of mice injected intracerebrally with STZ on day one and three and treated with vehicle for 21 days [195]. Other studies reported that a streptozotocin-induced experimental model of dementia showed a significant decrease in the brain GSH level, indicating neuronal damage due to oxidative stress [196].

The mechanisms by which STZ induces oxidative stress in the brain are not fully understood. It has been reported that brain slices from ICV STZ rats shows reduced glucose consumption from incubation medium comparing with control rats leading to hyperglycemia-like condition in brain [145]. Furthermore, there was increase in extracellular concentration of glucose in the brain of ICV STZ injected rats [195]. This may attribute to increase in nonenzymatic glycosylation of proteins and glucose auto-oxidation leading to oxidative stress and cellular damage [197]. The disturbance of glucose metabolism coupled with reduced activity of cytochrome oxidase leads to increase the production of ROS by mitochondria.This free radical generation leads to decreased antioxidant enzymes like GSH [195] which is indicated by a reduction in GR level. Also, ROS can be produced by microglia, which are activated by ICV STZ injection [197].

This study shows that ICV STZ injection in rats can cause the progressive deterioration of brain functions due to oxidative stress, so, it has been postulated that it may provide a relevant model of sporadic AD.

In the present study, the expression of GR in the hippocampus significantly increases when the doses of 5 mg/kg, 10 mg/kg and 20 mg/kg atorvastatin orally are given after injection of 3 mg/kg streptozocin intrathecally compared with the group administered 3mg/kg intrathecal streptozocin only. These findings were in accordance with the results of previous studies that showed atorvastatin had potential antioxidant effect [198]. Tramontina A. C. *et al*.(2011) reported that ICV-STZ injection reduced the total content of GSH in hippocampal slices and both simvastatin and pravastatin were capable of reversing this condition by preventing the effect of STZ on glutathione content [199].

The possible explanation for upregulation of GSH system in atorvastatin treatment may be related to stimulation of the transcription factor Nrf2, Nuclear Factor (erythroid-derived 2)-like 2, by statins in several experimental systems, including cultured neurons [200]. The Nrf2 mediated the expression of γ-glutamylcysteine synthetase, a key enzyme in GSH synthesis [201], which leads to increase its synthesis [202].

This study indicates that atorvastatin significantly increase the expression of GR after intrathecal STZ injection in a dose-dependent manner. As the doses increased from 5 to 10 to 20 mg/kg atorvastatin, the expression of GR in the hippocampus increased.

4.5. Effects of Atorvastatin and streptozocin on neuronal Nitric Oxide Synthase (nNOS)

Nitric oxide molecule ($NO^{\bullet}$) is a little reactive radical produced by three enzymes: endothelial nitric oxide synthase (eNOS), neuronal nitric oxide synthase (nNOS) and the inducible or inflammatory form expressed by macrophages (iNOS).The nitric oxide functions as a signaling molecule in the

vascular system. It is reported that NO molecules induce a vasodilatation effect by improving blood flow locally. Immediately after an ischemic insult or brain damage, eNOS is activated and exerts a protective vasodilation effects that enhance blood flow. Meanwhile, ischemic insults excessively activate the constitutively expressed nNOS and induce the expression and activation of iNOS, which lead to oxidative damage that caused by overproduction of ($NO^{\bullet}$) by these two enzymes [203].

In the current study, when neuronal nitric oxide synthase marker is used, there is a non-significant decrease in the expression of this marker in the hippocampus of rats treated with a 10 mg/kg atorvastatin orally compared with the control and 5 mg/kg atorvastatin groups. This disagree with study of de Oliveira C.V. *et al*. (2013), in which they found a decrease in NO content in the adult male rats cerebral cortex following 10 mg/kg of atorvastatin treatment for seven days or withdrawal at 24 hr. after the last atorvastatin administration [204] .This discrepancy could be due to methodological differences, where Oliveira C.V. *et al*. measured total NO content that produced from all the NOS isoforms in the rat's cerebral cortex.

The present study shows a significant decrease in nNOS expression in the group administered 20 mg/kg atorvastatin when compared with control, 5 mg/kg and 10 mg/kg atorvastatin treated groups. The possible explanation comes from the fact that statins down-regulate activity for both neuronal NOS and inducible NOS isoforms [205], which are the predominant NOS isoforms in the brain [206].

The findings of the present study show that a significant increase in the expression of nNOS in the hippocampus of rats administered 3 mg/kg streptozocin is agreed with previous study that found there was an increased in NO levels in the hippocampus of rats submitted to ICV- STZ injection [207].

Rai S. *et al* (2013) found that STZ (ICV) increased the nNOS mRNA and protein expression in hippocampus and cortex of adult male rats [208]. Thus, increased free radical generation, nNOS and iNOS gene expression leading to formation of proinflammatory cytokines, by activation of microglia and astrocyte, which is an important pathophysiological component of Alzheimer's disease [209].

The possible mechanism explained this increase in nNOS may be related to the fact that STZ caused an increase in Ca^{2+} level in cortex and hippocampus [210] that mediate the binding of calmodulin to the nNOS, which in turn increase the activity of this enzyme [211]. The High levels of NO produced from nNOS give rise to energy depletion, due to inhibition of mitochondrial respiration and inhibition of glycolysis [154].Also, it is contributed to excitotoxicity, probably via peroxynitrite production, that can cause oxidative damage, nitration, and S-nitrosylation of biomolecules including proteins, lipids, and DNA [154].

The results of this study demonstrate that a significant reduction in nNOS level in the hippocampus of rats treated with 10 mg/kg and 20 mg/kg atorvastatin orally after intrathecal injection of 3mg/kg streptozocin compared with group injected 3mg/kg streptozocin alone. In regard to atorvastatin, the present study findings are in accordance with the result of Moro M.A. *et al* (2004) in which they documented that treatment with atorvastatin may lead to decrease overproduction of NO by nNOS and iNOS, after the onset of the ischaemic brain injury, through down-regulating both enzymes [212].

4.6. Effects of Atorvastatin and streptozocin on lipid profile

The effects of treated groups on lipid profile in the present study demonstrates that atorvastatin reduced total cholesterol, LDL, TG and does not change serum HDL level after 30 days of treatment. While, streptozocin have no effect on lipid profile.

A number of large clinical trials, in regards to statin, have demonstrated their clinical usefulness for counteract hyperlipidemias, the major cause of atherosclerosis which, in turn, is a common pathogenetic mechanism for cardiovascular events, such as myocardial infarction, stroke, and sudden cardiac death [213, 214]. The result of the present study shows that atorvastatin treatment improves lipid profile which agreed with other studies [215, 216]. The mechanism involved is most likely attributed to the ability of atorvastatin to impair cholesterol synthesis via inhibiting the enzyme HMG-CoA reductase, which is the rate limiting step in cholesterol biosynthesis. This leads to both, decrease circulating LDL-C concentrations and increase their uptake and extraction from the blood by induce LDL-receptor expression on the hepatocyte cell surface. The overall lipid lowering effect include increase uptake and degradation of LDL-C, inhibition of LDL-C oxidation, reduction in cholesterol accumulation and esterification and decreases lipoprotein secretion and cholesterol synthesis [217, 218]. Atorvastatin and rosuvastatin can also decrease TG in some patients, perhaps by reducing the rate of very low density lipoprotein synthesis and increasing its clearance [219].

In the present study, serum level of cholesterol, LDL and TG significantly decreases with the increase in the atorvastatin dose from 5 mg/kg to 10 mg/kg to 20 mg/kg, these results were agreed with those found by Tousoulis D. *et al*. (2013) showed that, after 4 weeks of atorvastatin treatment, people taking a dose of 40 mg/day atorvastatin exhibited more

reduction in the serum level of cholesterol, LDL and TG than those treated with a 10 mg/day [220]. Fujita M. *et al.* (2007) showed that there is a greater significant reduction in serum LDL level when a 10 mg/ day of Atorvastatin dose was administered than the 5 mg/day of atorvastatin dose. On the other hand, they displayed that the small dose (5 mg) of atorvastatin significantly decreased triglyceride concentrations, but the higher (10 mg) dose of the statin did not further significantly decrease TG [221].

In this study, HDL level does not change significantly with different doses of atorvastatin treatment consistent with previous studies [219, 222]. Asztalos B.F *et al.* (2002) examined the effects of atorvastatin treatment in a period lasted for 12 weeks (4-week periods of 20 mg/day then 40 mg/day, then 80 mg/day) on HDL subpopulation profile of coronary heart disease patients, their study demonstrated that there were a non-significant increases in HDL-C levels compared with placebo treatment [223].In contrast, Athyros VG *et al.* (2004) reported a significant increase in HDL level after treatment with a titration dose of atorvastatin (10-80 mg/day) [224].

AD is a neurodegenerative disorder associated with cognitive and behavioral dysfunction and this may cause dementia in the elderly. It is generally believed that AD is multi-etiologic. Many kinds of stresses could effect brain neuron and impair its normal function. For example, environmental, metabolic, and genetic factors may cause brain lesions through various pathways.

Previous studies reported that abnormal cholesterol metabolism is one of the risk factors of AD. Epidemiological studies have linked elevated plasma cholesterol and lipoprotein levels with AD development [225, 226]. The findings of this study indicates that administration of 3 mg/kg streptozocin intrathecally shows non significant changes in the cholesterol, LDL, TG and

HDL level compared with control values. These results are in agreement with those found by Tramontina A. C. *et al*. (2011) who did not find any changes in total cholesterol and triglyceride serum level in ICV-STZ treated rats [199].

On the other hand, Adewole S.O and Ojewole J.A (2009) reported that Serum total cholesterol, triglycerides and LDL cholesterol were significantly elevated in STZ-treated rats Group as compared with control Group. Similarly, HDL cholesterol was significantly reduced in STZ-treated group diabetic rats [227]. The most possible cause of this diversity between the present study and Adewole S.O and Ojewole J.A (2009) is that the latter one used intraperitoneal injections of 70 mg/kg streptozotocin, while intrathecal injections of 3 mg/kg streptozocin used in the present study.

The results of the current study shows that a significant improved in lipid profile in the groups of rats treated with 5, 10 and 20 mg/kg atorvastatin after injection with 3 mg/kg streptozocin intrathecally when compared with control and group administered streptozocin only, in regard to the effect on serum LDL-cholesterol, these results in agreement with other previous studies that showed after the administration of atorvastatin (80 mg) for 52 weeks or simvastatin (40 mg) for 26 weeks a reduction of LDL-c of 50–54% in AD patients was observed [228, 229]. Fen L. *et al.* (2010) mentioned that treatment with 15 mg/kg atorvastatin for three and six months significantly decrease total cholesterol, LDL and TG serum level in the rats exhibited tau phosphorylation and increasing in Aβ level in their brains after consumption of high cholesterol diet [230].

In contrast, Kurata T. *et al.* (2011) reported that a non -significant changes in serum triglycerides, total cholesterol, HDL or LDL levels at 10 , 15 and 20 months of age in the amyloid precursor protein (APP) transgenic (Tg) mice groups after treated with (30 mg/kg) atorvastatin and (3 mg/kg)

pitavastatin [231]. This discrepancy could be related to methodological differences that Kurata T. *et al.* used of amyloid precursor protein transgenic mice which may affect the results, while the present study used streptozocin-induced model of AD in rats.

The results of this study shows that there are greater significant improvements in the lipid profile as the dose of atorvastatin increased from 5 to 10 and to 20 mg/kg which means the effect of short term atorvastatin treatment on lipid profile in this study is in a dose dependent manner.

4.7. Conclusions: according to the results obtained from this study, one can conclude the following:

- Administration of streptozocin intrathecally may yield a model of Alzheimer's disease indicated by brain damage which in turn improved by atorvastatin treatment.
- Present study demonstrates that atorvastatin exerts its neuroprotective effects in a dose dependent manner.
- Atorvastatin would be used for improving lipid profile. While, intrathecal injection of streptozocin has no effect on lipid profile.

4.8. Recommendations for future work:

- Longer duration with larger number of rats treated with atorvastatin doses to help further understand the nuroprotective mechanisms of atorvastatin in a model of Alzheimer's disease.
- Using of adult female rats and other generations of statins with different doses and using other markers of Alzheimer's disease to see their effects on the brain.

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الخلاصة

المقدمة:

يعتبر الستاتين،بالآضافه الى قابليته على تخفيض الدهون، عامل مفيد في حماية الجهاز العصبي في بعض الحالات السريرية مثل اصابات الدماغ، السكتة الدماغية، نقص التروية، الصرع ومرض الزهايمر.

الهدف:

تم تصميم هذه الدراسه للآسباب التالية:

1- دراسة تأثير جرعات فموية مختلفة من أتورفاستاتين على الفئران الذكور البالغة الطبيعية من خلال دراسة الدليل الكيميائي النسجي المناعي في منطقة قرن امون.

2- دراسة تأثير جرعات فموية مختلفة من أتورفاستاتين على الفئران الذكور التي تمثل نموذج من مرض الزهايمر الناجم عن الحقن النخاعي لمادة الستربتوزوسين بجرعة ٣ ملغ / كغم عبر دراسة الدليل الكيميائي النسجي المناعي في منطقة قرن امون.

المواد وطرق العمل:

تم استخدام ثمان وأربعين ذكر من فئران ويستار البالغة (وزنها ٢٠٠-٢٥٠ غم) في التجربة. تم تقسيم الحيوانات إلى ٨ مجموعات عشوائيا كل مجموعة تحتوي على ٦ حيوانات على النحو التالي: المجموعة ١: الحيوانات كانت تعطى محلول الملح فمويا لمدة ٣٠ يوما وتكون بمثابة المجموعة الضابطة. المجموعة ٢ : تم اعطاءهم جرعة واحدة من ٣ملغ / كغم ستربتوزوسين كحقنة نخاعية متبوعة باعطاء محلول الملح فمويا لمدة ٣٠ يوم. المجموعة ٣: تم اعطائهم حقن نخاعي لمادة الستربتوزوسين(٣ملغ / كغم) كجرعة واحدة، في نفس اليوم، تم اعطاء ٥ملغ / كغم / يوم من دواء الأتورفاستاتين لمدة ٣٠ يوما. في حين، تم اعطاء الفئران في المجموعة ٤ و ٥ نفس علاج المجموعة الثالثة ولكن مع ١٠ ملغ / كغم و ٢٠ ملغ / كغم من الاتورفاستاتين على التوالي. المجموعة ٦ كانت تعطى ٥ ملغ / كغم / يوم من الأتورفاستاتين لمدة ٣٠ يوما. بينما مجموعة ٧ و ٨ كانت تعطى أتورفاستاتين فمويا لمدة ٣٠ يوما بجرعة ١٠ ملغ / كغم و ٢٠ ملغ / كغم على التوالي. في اليوم ٣١ ،تحت التخدير، تم تشريح جميع الحيوانات في هذه الدراسة.

النتائج:

بينت نتائج هذه الدراسة زيادة معنويه كبيرة في اظهارال GFAP في قرن امون الجرذان التي اعطيت حقنه نخاعية من الستربتوزوسين عند مقارنتها مع المجموعة الضابطه. في حين، لوحظ انخفاضا معنويا كبيرا في ذلك البروتين في المجموعة المعالجة ب ٢٠ ملغ / كغم من الأتورفاستاتين وحده عند مقارنته بالمجموعة الضابطة، كذلك لوحظ انخفاضا معنويا في ذلك البروتين في المجموعة التي اعطيت ٢٠ ملغ / كغم الأتورفاستاتين + ستربتوزوسين كحقنه نخاعيه واحدة عند مقارنته بالمجموعة التي اعطيت ستربتوزوسين وحده. أظهرت الدراسة حالة المضادات للأكسدة ،التي يمثلها انزيم GR،

اظهرت النتائج زيادة معنوية كبيرة في اظهار GR في قرن آمون للفئران التي عولجت بجرعة ٢٠ ملغ / كغم من الاتورفاستاتين وحده (عند مقرنتها مع المجموعة الضابطة)، والمجموعات التي اعطيت ٥ و ١٠ و ٢٠ ملغ / كغم أتورفاستاتين بعد الحقن النخاعي لمادة الستربتوزوسين (عند مقارنتها مع المجموعة التي اعطيت ستربتوزوسين وحده). في حين، لوحظ انخفاضا معنويا كبيرا في هذا الانزيم في المجموعة التي اعطيت مادة الستربتوزوسين وحدها عند مقارنتها مع المجموعة الضابطة. لوحظت زيادة معنويه كبيرة في التعبير عن انزيم nNOS في قرن امون الجرذان التي اعطيت حقنة نخاعية من مادة الستربتوزوسين عند مقارنتها مع المجموعة الضابطة. في الوقت نفسه، لوحظ انخفاظ معنوي كبير في هذا الانزيم في المجموعة التي تلقت علاج الاتورفاستاتين وحده بجرعة ٢٠ ملغ / كغم (عند مقارنتها مع المجموعة الضابطة) ، والمجموعات المعالجة مع ١٠ و ٢٠ ملغ / كغم أتورفاستاتين جنبا إلى جنب مع الحقن النخاعي لمادة الستربتوزوسين (عند مقارنتها مع المجموعة التي اعطيت ستربتوزوسين كحقنة نخناعية واحدة).

أضهرت نتائج هذه الدراسة ان هناك تحسن معنوي في مستويات الكوليستيرول، TGوLDL عند زيادة جرعة الأتورفاستاتين من٥ الى ١٠ الى ٢٠ ملغ / كغم، ولكن لايوجد اي تغير معنوي في مستوى ال HDL عند اعطاء الاتورفاستاتين بكل جرعه.

أظهرت الدراسة انه لايوجد اي تغير معنوي في مستويات الدهون عند الحقن النخاعي لمادة الستربتوزوسين.

الاستنتاج:

وفقا للبيانات التي تم الحصول عليها خلال هذه الدراسة يمكننا ان نستنتج:

١) الحقن النخاعي لمادة الستربتوزوسين قد تؤدي الى عمل نموذج من مرض الزهايمر المستدل عليه من خلال التلف الدماغي الذي بدوره يحسن عن طريق العلاج بدواء الاتورفاستاتين.

٢)توضح الدراسة أن أتورفاستاتين يعمل عل حماية الجهاز العصبي بطريقة تعتمد على الجرعة المعطاة.

٣)يمكن اعتبار الأتورفاستاتين العلاج الفعال في تحسين مستوى الدهون في الدم. في حين، الحقن النخاعي لمادة الستربتوزوسين ليس له تأثير على مستوى الدهون في الدم.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

الجامعة المستنصرية

كلية الصيدلة

تأثير دواء الاتورفاستاتين والستربتوزوسين على الدليل الكيميائي النسيجي المناعي في منطقة قرن امون لذكور الجرذان البالغة

رسالة مقدمة الى فرع الأدوية والسموم والى لجنة الدراسات العليا في كلية الصيدلة /الجامعة المستنصرية كجزء من متطلبات الحصول على شهادة الماجستير في علوم الصيدلة (الأدوية والسموم)

من قبل

الصيدلانية

الاء عادل عزيز الهنداوي

(بكلوريوس صيدلة ٢٠٠٩)

باشراف

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