Ameliorating Effect of Memantine Hydrochloride Treatment on Cisplatin-Induced Toxicity with Special Reference to the Neuro-, Nephro- and Hepato-toxicity in Male Mice

By
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Supervised By

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بسم الله الرحمن الرحيم

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ * خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ * اقْرَأْ وَرَبُّكَ الْأَكْرَمُ * الَّذِي عَلَّمَ بِالْقَلَمِ * عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمْ

صدق الله العظيم

سورة العلق، الآيات (1-5)

Dedication

*** To the memory of my dear father***

*** To the symbol of kindness my dear mother***

To those who:

*** Taught me the letters, the words, the sciences***

*** Supported me during the hard times;**

My dear husband Dr. Luqman Kh. Rasool and my lovely daughter Zhilia

Abstract

Cisplatin is one of the most active cytotoxic drugs and a widely utilised anti-cancer agent which is used to treat various types of cancer. The use of cisplatin is limited due to its main side effects, including, hepatotoxicity, nephrotoxicity, neurotoxicity and hematotoxicity which may arise from free radical damage. There is evidence to show that memantine reduces oxidative stress-induced damage. This study was conducted to explore the possible protective role of memantine by pre-treatment with two different therapeutic doses 5mg/kg and 10mg/kg of orally administered memantine, which is used as an agent to minimise the toxic side effects of an injection of 4 mg/kg cisplatin with special references to the kidney, liver, and neurobehavioral alterations in male mice. This study consists of 3 main experiments:-

In the first experiment, the acute toxic dose was measured for both drugs cisplatin and memantine separately by up and down method. In the second experiment, main adverse effects of cisplatin which are nephrotoxicity and hepatotoxicity were studied by measuring the oxidative stress markers, histopathological examination of various tissues in the body and immunohistochemical staining of different organs for both myeloperoxidase and nicotinic acetylcholine receptors.

Those mice injected intraperitoneally with cisplatin showed significantly increment levels of alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, alkaline phosphatase, serum creatinine; malondialdehyde, creatine kinase, serum acetylcholine esterase, up-regulation score of myeloperoxidase and nicotinic acetylcholine receptors with highly fluctuation levels of glutathione, glutathione transferase, superoxide dismutase, catalase, glutathione peroxidase, dopamine, serotonin, sodium, calcium, and potassium contents. Moreover, histopathological examination of kidney, brain, skeletal muscle, liver, lungs and testis tissue reflected marked injury.

Blood examinations in those mice treated with cisplatin showed a significant increased level of white blood cell count and a significant decline in the level of red blood cell count, packed cell volume and hemoglobin, these measurements are the main criteria for hematotoxicity, the third experiment

conducted to explore the role of memantine in antagonizing neurobehavioral toxicity of cisplatin. Weekly neurobehavioral investigations were studied for 30 days by using the following battery of tests: open field activity, negative geotaxis, hole-board test, and swimming test. Those mice treated with cisplatin showed significantly decreased ability to perform the neurobehavioral tasks and decrease weight gain in contrast to those mice in combination treated group of cisplatin with memantine hydrochloride exhibited a significantly improved ability and normal weight gain pattern in mice thus memantine hydrochloride offered partial protection at dose 5 mg/kg while offered complete protection at dose 10 mg/kg.

In conclusion, findings from this study revealed the crucial role of memantine to antagonize the main adverse effects of cisplatin in trends to nephrotoxicity, hepatotoxicity and neurotoxicity.

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Nadia

Declaration Form

I hereby confirm that:

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Declaration by Supervisor

This is to confirm that:

- The research conducted and the writing of this thesis was under our supervision;
- Supervision responsibilities are adhered to

Assistant Professor: Dr. Banan Khalid Al -Baggou

4/04 / 2019

Committee certification

I certify that a Thesis Examination Committee has met on / / 2019 to conduct the final examination of (Nadia Abdulkareem Salih) on her thesis entitled" Effects of memantine hydrochloride on cisplatin-induced toxicity with special reference to the neuro-, nephro- and hepato-toxicity in mice. The Committee recommends that the student be awarded the Degree of Doctor of Philosophy.

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In view of the available recommendation, I forward this thesis for debate by the examining committee.

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Date: / /2019

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List of Abbreviations

Abbreviation	Details
RBCS	Red blood cells
WBCS	White blood cells
G	Group
Pt	platinum drugs
µg/dl	Microgram/deciliter
mg/dl	Milligram/deciliter
%	Percentage
PCV	Packed cell volume
Hb	Hemoglobin
ml	Milliliter
α	Alpha
β	Beta
γ	Gama
δ	Delta
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
BUN	Blood urea nitrogen
CK	Creatine kinase
Scr	Serum creatinine
LD_{50}	Median lethal dose
EDTA	Ethylenediaminetetraacetic Acid

FDA	Food and drug administration
GSH	Glutathione
GPx	Glutathione peroxidase
H2O2	Hydrogen peroxide
ROS	Reactive oxygen species
RNS	Reactive Nitrogen Species
SOD	Superoxide dismutase
DRG	Dorsal Root Ganglion
PBS	Phosphate Buffer Saline
rpm	Rotation per minute
Abs	Antibodies
ELISA	Enzyme-linked Immunosorbent assay
GABA	Gamma amino butyric acid
NMDAR	N-methyl-D-aspartate receptor
Glutamate	GLU
ANOVA	Analysis of variance
Bcl-2	B-cell lymphoma 2
CP	Cisplatin
SR	Sarcoplasmic Reticulum
Min	Minutes
D.W	Distilled water
UTI	Urinary tract infection
GFR	Glomerular filtration rate
nAChRs	nicotinic acetylcholine receptors
MPO	Myeloperoxidase
DNA	Deoxyribonucleic acid

Chapter One

INTRODUCTION

Cisplatin (Cis-diamminedichloroplatinum) is a commonly used cytotoxic agent, water-soluble member of the platinum anticancer drugs; an efficient platinum derived alkylating drug that acts against both of resting and proliferating cells, with a broad spectrum of activity against solid malignant tumors, including germ cell, ovarian, endometrial, gastric, testicular, cervical, urothelial, head and lung cancer (Abdelmeguid et al., 2010, Bergstrom et al., 1999). Nonetheless, its clinical use is restricted because of numerous adverse reactions, for example, nephrotoxicity, hepatotoxicity, myelotoxicity, ototoxicity, and neuropathy. Its anticancer impact is acquired by few pathways, including the generation of DNA adducts and reactive oxygen species (ROS) (Boulikas and Vougiouka, 2003).

The toxicity of cisplatin is dose-dependent caused by cumulative action of the drug, where its accumulation produces clear necrotic changes inside the tissues of predisposed organs (Agarwal et al., 2008a, Bergstrom et al., 1999).

The liberation of ROS and nitrogen species is among the proposed mechanisms responsible on cisplatin toxic effects through the injury caused by the generation of free radicals and diminished antioxidant defense system, which results in oxidative harm in different tissues and reactions, which cause cell dysfunction (Kelland, 2007, Circu and Aw, 2010). Oxidative stress is an important contributor to the pathogenesis of various pathological conditions including inflammation and drug toxicity (Agarwal et al., 2008a, Agarwal et al., 2008b).

The body has multiple mechanisms as body innate defense systems which preserve the structure and function of cellular molecules against ROS induced damage (Valko et al., 2004, Han et al., 2001). However, the body barrier may not be sufficient for shielding it from serious oxidative harm. Hence, certain exogenous amount of antioxidants and drugs are necessary to maintain an acceptable level of antioxidants to keep a balance between generation of ROS and the body defense system. Until now, a large number of studies have been focused on ways of preventing cisplatin side effects using herbal products (Al-Badrany and Mohammad, 2007, Albuquerque et al., 2009). This study conducted to explore the ultimate protective role of memantine as a newly tried agent against significant adverse reactions of cisplatin; nephrotoxicity, hepatotoxicity, hematotoxicity, and neurobehavioral toxicity, that can be advantageous during treatment with platinum antineoplastic agent in those who suffer from cancer.

This study create the base for other researchers to discover other critical areas of a newly tried agent such as memantine in combination with cisplatin, this area has never been studied before with anticancer drugs, this study focused on a new indication of memantine hydrochloride, in last years all works on cisplatin focused on use of antioxidants agents as a protective agent for diminishing side effects of cisplatin, but this work idea is different, by pulling attention to new probable clinical use of highly effective and widely used drugs like memantine by depending on its mechanism of action as glutamate antagonist and N-methyl-D-aspartate (NMDA) receptor antagonist.

1.1. Aim(s) of the study

1. Exploring the possible use of memantine hydrochloride, an Alzheimer's disease remedy, as an agent to minimize the toxic side effects of cisplatin on blood values and some tissues and organs with special reference to the neurobehavioral, liver, and kidney alterations

2. Elucidating the possible mode of action of memantine hydrochloride as a neuroprotective agent in cisplatin-induced neurotoxicity in mice.

3. Investigates the effect of memantine hydrochloride on histopathological changes in the brain, skeletal muscle, liver, kidney, testes, heart, and lung.

Chapter two

LITERATURE OF REVIEW

2.1. Cancer

Cancer is a malignant growth among the most spread and life-costing illnesses in the present society, described by uncontrolled division of cells, which have lost their ability for differentiation (Abdel Moneim et al., 2014, Abdelmeguid et al., 2010, Bergstrom et al., 1999). Localized benign tumors, not spread to other parts of the body, while the malignant tumor is able to invade nearby tissues and forming metastasis (Albuquerque et al., 2009, Boulikas and Vougiouka, 2003).

There are in excess of 100 malignancy types, which could be categories in to carcinoma (tumor of the epithelial tissue), sarcoma (tumor of the connective tissue), leukemia (tumor of the blood-forming tissue such as the bone marrow and makes extensive quantities of blood cells to be created), lymphoma and myeloma (tumor of the cells of the immune system), central nervous system cancers (tumor of the brain and spinal cord)(Boulikas and Vougiouka, 2003, Abdel Moneim, 2014).

The classical treatment strategies for cancer include a combination of surgery, radiotherapy, and chemotherapy. For chemotherapy, there is an extensive number of antitumor agents (Boulikas, 2004). They can be classified depending on their chemical structure, mechanism of action, origin and cellular targets (Brock et al., 2012, Carrasco et al., 2007).

2.1.1. Classification of chemotherapeutic agents

• Alkylating and metallating agents – react directly with nucleophilic groups of DNA.

- Nitrogen mustards (melphalan, chlorambucil, ifosfamide).

- Nitrosoureas (dacarbazine, carmustine).

- Other alkylating agents (busulfan).

- Platinum complexes (cisplatin, carboplatin, oxaliplatin).

• Antitumor antibiotics and derivatives – DNA intercalators, generation of ROS, topoisomerase II poisons.

- Anthracyclines (dactinomycin, doxorubicin).

- Synthetic analogs (mitoxantrone).

- Glycopeptides (bleomycin).

• Antimetabolites – inhibit the enzymes, involved in DNA synthesis; S-phase specific agents:-

- Dihydrofolate reductase inhibitors (methotrexate).

- Ribonucleotide reductase inhibitor (hydroxycarbamide).

- Pyrimidine antagonists (gemcitabine, 5-fluorouracil).

- Purine antagonists (6-mercaptopurine, 6-thioguanine).

• Antitumor agents from plant origin and derivatives (cell cycle specific agents):-

- Topoisomerase inhibitors – podophyllotoxins and camptothecins.

- Tubulin polymerization inhibitors (Vinca alkaloids, cryptophycins).

- Tubulin depolymerization inhibitors – taxanes.

• Hormone-based therapies – used for hormone-dependent cancers:-

- Glucocorticoids, estrogens, progestins, and androgens (fosfestrol).

- Antiandrogens (flutamide).

- Antiestrogens (tamoxifen).

- Aromatase inhibitors (anastrozole, letrozole).

• Inhibitors of signaling pathways - protein kinase inhibitors and others.

• Other cytotoxic agents (Asparaginase, As2O3, etc.)(Brock et al., 2012, Abdel Moneim, 2014, Bennett et al., 1980, Boulikas and Vougiouka, 2003, Frezza et al., 2010).

2.1.2. Platinum -based complexes in cancer therapy

Discovery of cell division inhibition in E.coli cultures, caused by the platinum species, formed as electrolysis products during the experiments by Barnett Rosenberg in 1965 initiated the establishment of metal-based anticancer drugs in medicinal chemistry. Three platinum complexes (cisplatin, oxaliplatin and carboplatin) are utilized widely in many of the oncological treatment regimens worldwide (figure 2.1) (Galanski, 2006, Kelland, 2007, Lee et al., 2007, Brown et al., 2010).

Platinum drugs are a piece of the first line chemotherapy in twelve neoplasms (testicular cancer, ovarian cancer, bladder cancer, small cell lung cancer, non-small cell lung cancer, head and neck cancer, esophageal cancer, thymoma, osteogenic sarcoma, cervical cancer and colorectal cancer (Abdel Moneim, 2014, Ali et al., 2007).

Figure 2.1: Shows the chemical structure of Pt (II) complexes (Cisplatin, Carboplatin, Oxaliplatin)(Zhu et al., 2013)

Besides the therapeutic effects of platinum drugs, they have many side effects. The ability to minimize these toxic side effects is a critical point for the success of cancer chemotherapy (Chan et al., 2009b, Akman et al., 2015, Ali et al., 2008a).

2.1.3. Cellular mechanism of Platinum antineoplastic agents

Medical researches have illustrated the mechanism of the action of chemotherapeutic agents, basically inhibiting metabolic pathways necessary for cell division (Takizawa et al., 1990, Vaissiere et al., 2009). Molecular and genetic approaches have enhanced the understanding of cell signaling networks that regulate proliferation and survival. Antineoplastic agents destroy malignant mass via enlistment of apoptosis, which is mediated by the activation of various signal transduction pathways (Fearon et al., 2012)(figure 2.2).

This impact discussed the inhibition of DNA synthesis and repair that result in cell cycle switch off and arrest consequently; apoptosis will be induced. Oxidative stress can disrupt normal biological functions, ongoing information recommend that cisplatin also induces ROS that triggers cell death (Unger et al., 2005, Pu et al., 2001).

Cell passing happens upon simultaneous actuation of a few flagging pathways, the arrangement of ROS relies upon the convergence of cisplatin and the term of exposure (Qiao et al., 2017). The intracellular redox homeostasis is maintained by the thiol group (-SH) containing atoms. Under certain conditions a thiol group may lead to the formation of thiyl radicals that in turn can interact with molecular oxygen, therefore generating ROS (Rogawski and Wenk, 2003, Zhang and Zhang, 2004) (figure 2.2).

Multiple mechanisms result in repair inhibition: (a) directly by free radical or (b) indirectly by lowering the level of reduced glutathione. Normally, every cell has a harmony among cancer prevention agents and free radicals. At the point when this parity changed; they actuate lipid peroxidation, exhaustion of the sulfhydryl groups, altered signal transduction pathways, altered calcium homeostasis, and DNA damage. This consequently results in aging and/or cancer (figure 2.2) (Ramoutar and Brumaghim, 2010, Rastghalam et al., 2014).

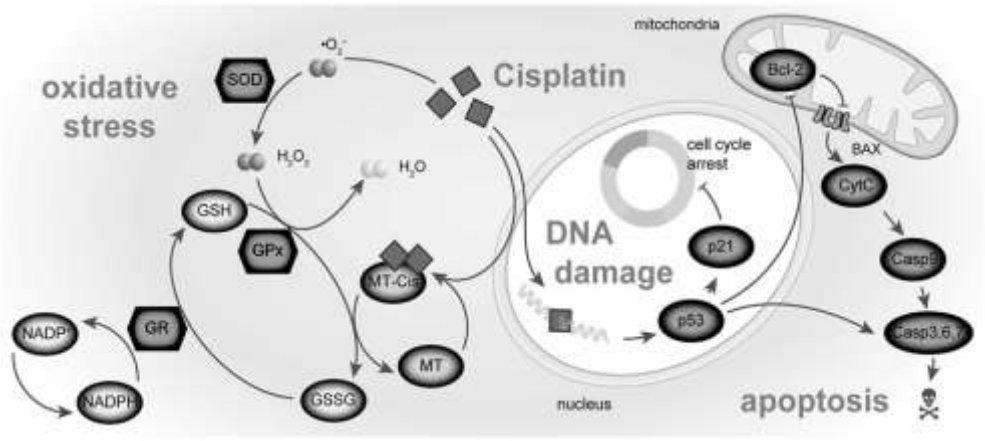

Figure 2.2: Shows the cellular Pathway of Platinum Antineoplastic Agents Cisplatin-induced DNA damage, oxidative stress and apoptosis (Gumulec et al., 2014).

2.2. Cisplatin (cis-diamminedichloridoplatinum)

Cisplatin (cis-diaminodichloroplatinum) was the first platinum-based antineoplastic agent, approved in 1978 by the food and drug adminstartion (FDA) and it is a standout amongst generally utilized in chemotherapeutic regimens for solid tumors of the ovary, skin, lung, testis, head, and neck (Zhu et al., 2014, Zhong et al., 2008).

A characteristic reaction of cisplatin is neurotoxicity, leading to peripheral neuropathy. This portion subordinate lethal neuropathy is related with distal paresthesias influencing hands and feet, gathering of cisplatin in dorsal root ganglion (DRG) neurons as platinum-DNA adducts are accepted to be among the essential mechanisms of neurotoxicity. High prevalence of neurotoxicity limits the chemotherapeutic efficacy of cisplatin (Yao et al., 2007, Yonezawa et al., 2006).

In 1965, during tests on the impacts of electric fields on cell development and division, Rosenberg and colleagues found that an electric flow conveyed between platinum terminals inhibited the expansion of Escherichia coli microscopic

organisms. This inhibitory impact was observed to be related to the arrangement of inorganic platinum complexes (Fearon et al., 2012, Yeung et al., 2007). Extra research demonstrated that the cis isomer of dichlorodiammineplatinum II (cisplatin) is a functioning inhibitor of cell division, while the trans isomer had no influence on cell growth manners (Zhong et al., 2008, Boulikas and Vougiouka, 2003). The antiproliferative activity of cisplatin has been attributed predominantly to the binding of cisplatin to adenine and guanine resulting in DNA-platinum adducts with the formation of intrastrand and interstrand cross-links (Townsend et al., 2003, Takizawa et al., 1992, Kudo et al., 2011, Lieberman, 2001). Cisplatin ties covalently and irreversibly to plasma proteins (over 90%). Cisplatin is dispersed in various organs and tissues with most noteworthy fixations in liver, prostate, kidney, and ovaries (Luo et al., 2008, Apak et al., 2007).

The primary objective of logical research concentrating on platinum edifices is to recognize aggravates that have predominant adequacy, diminished poisonous quality, absence of cross-opposition or improved pharmacological attributes as contrasted and the parent compound, cisplatin is as often as possible utilized in mix with one, two, three, or even four different medications, with positive outcomes. The expectation is that the medications will cooperate, creating synergistic or if nothing else added substance impacts in executing disease and cancer cells while producing no additional side effects (Johnsson and Wennerberg, 1999, Awad et al., 2012, Boulikas and Vougiouka, 2003, Yao et al., 2007).

2.2.1. Mechanism of action cisplatin

Cisplatin is broadly utilized in the cure of dense tumors, exerts an anti-tumor activity similar to classical alkylating agents. The drug then concentrated in the

tissues and can then interact with macromolecules, such as DNA, to form intrastrand adducts and interstrand cross-links (Yao et al., 2007, Takizawa et al., 1990). The aquated cisplatin ties with profoundly nucleophilic N-7 places of the purine bases guanine and adenine. Cisplatin can also tie to RNA and cell proteins. The real impact of cisplatin is to suppress cell replication and DNA union (Zhang et al., 2017, Takizawa et al., 1992). After administration, cisplatin is bound to plasma proteins. Almost a fourth of the controlled dose is discharged through the kidneys amid the initial 24 hours. The all-out cisplatin (free and bound) has a drawn-out half-existence of 2 to 3 days, it can stay bound to protein tissues for a significant lot of time. Cisplatin potentiates the sublethal damage induced by radiation and inhibits repair of potentially lethal damage (figure 2.3) (Brown et al., 2010, Boulikas and Vougiouka, 2003).

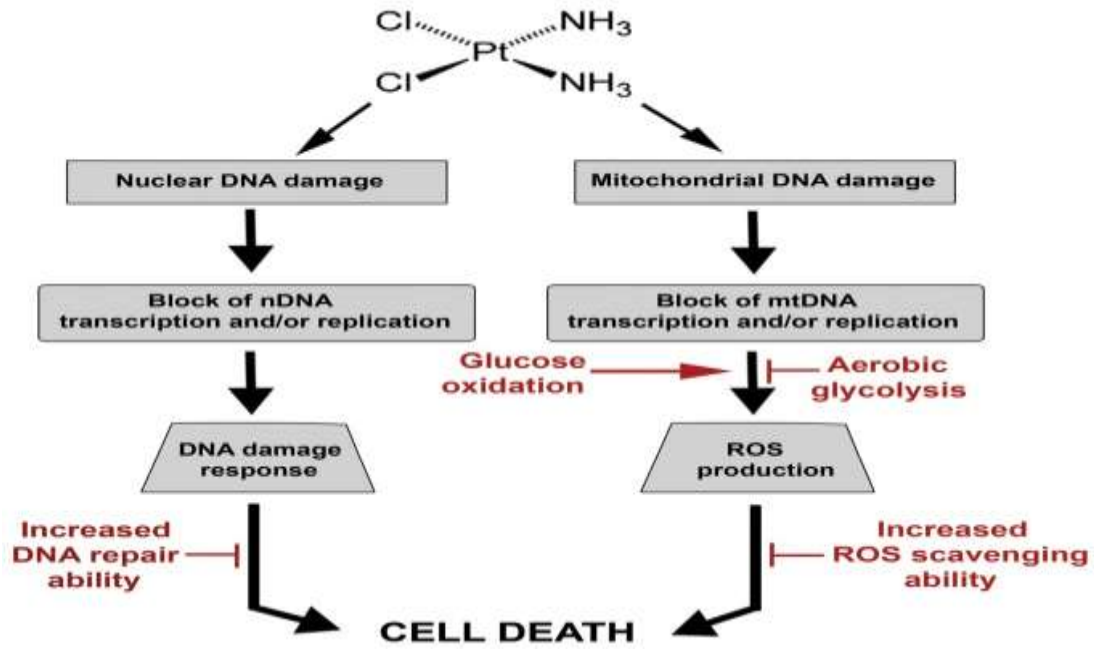

Figure 2.3: Shows schematic illustartion of the meachnism of action of cisplatin (Marullo et al., 2013)

2.2.2. Dosage and Administration

Cisplatin is directed by moderate intravenous infusion, causing encourage arrangement and lost intensity. The standard pharmaceutical dose in the treatment of ovarian tumors is 75 to 100 mg/m2 IV per cycle once at regular intervals (Arany and Safirstein, 2003, Boulikas and Vougiouka, 2003). In advanced bladder cancer, cisplatin ought to be controlled as a solitary specialist at a dose of 50 to 70 mg/m2 IV per cycle once every 3 to 4 weeks depending on the extent of prior exposure to radiation therapy and/or prior chemotherapy (Brown et al., 2010, Boulikas and Vougiouka, 2003).

2.2.3. Major side effects of cisplatin

1. Nephrotoxicity

Nephrotoxicity is a dose related and combined renal inadequacy, including intense renal failure. Renal harmfulness has been distinguished in 28% to 36% of patients inoculated with a solitary portion of 50 mg/m2. It is principal prominent during the second week after one dose and is showed by heights in blood urea nitrogen BUN and serum creatinine Scr, reduced serum magnesium, sodium and potassium levels. Renal injuriousness becomes more persistent and serious with continuous administration of the drug, injury of the renal function has been related to renal deep hurt. Nephrotoxicity was documented as the main renal response of cisplatin, happening in about one-third of the patient undergoing cisplatin treatment. Cisplatin nephrotoxicity is frequently occurring after 10 days of usage and is

revealed as a reduction in glomerular filtration rate (Boulikas and Vougiouka, 2003, Ciarimboli et al., 2005, Kwak et al., 2007).

The pathophysiological premise of cisplatin nephrotoxicity is investigated by the presentation of tubular cells to cisplatin initiates complex flagging pathways that lead to tubular cell damage (Keum et al., 2008, Klaunig et al., 1998). Cisplatin may also persuade damage in renal vasculature and result in reduced blood flow and ischemic damage of the kidneys, contributing to a decline in glomerular filtration rate. These events, together, culminating in the loss of renal function during cisplatin nephrotoxicity (Kwak et al., 2007, Lee et al., 2007, Wang and Lippard, 2005)

Introduction of tubular cells to cisplatin triggers compound signaling pathways that result in tubular cell injury and death, a series of an inflammatory response is motivated, additional worsening of renal tissue damage. Cisplatin consequences are diminished bloodstream and ischemic damage of the kidneys and reduction in glomerular filtration rate. These events, totally lead to damage of the renal function, triggering acute renal failure (figure 2.4) (Stewart and Bolt, 2012, Huang et al., 2001a).

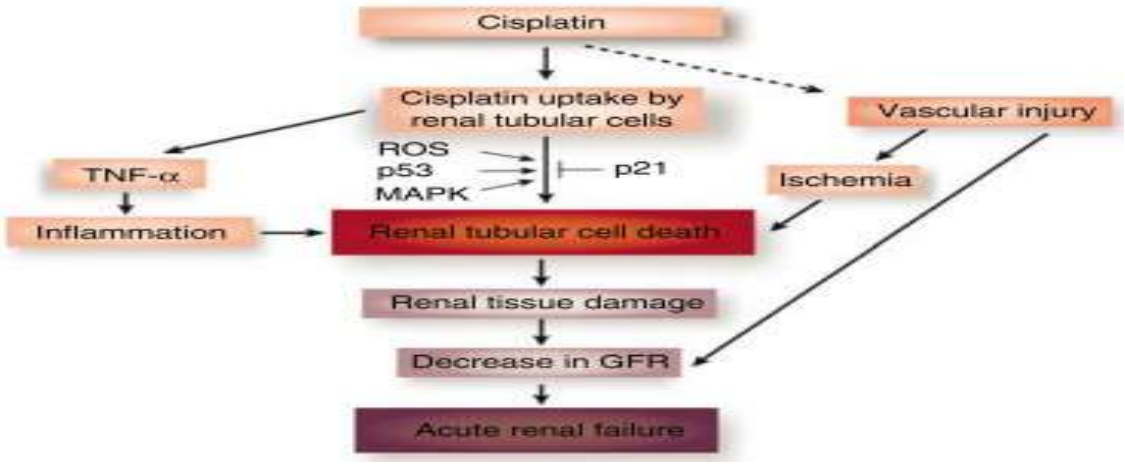

Figure 2.4: Shows the pathway of cisplatin induced nephrotoxicity(Pabla and Dong, 2008)

2. Hepatotoxicity

One of the conceivable mechanisms in charge of cisplatin toxicity is through oxidative stress damage and suppression of the antioxidant defense system, which creates an irregularity between the oxygen-determined radicals and endogenous levels of both enzymatic and non-enzymatic cell reinforcements prompting oxidative stress (Guan et al., 2017, Hsu et al., 2007, Al-Malki and Sayed, 2014, Chakravarthi et al., 2006). The precise appliance of hepatotoxicity induction is not completely understood, it is well-thought-out to be related to oxidative damage, during cisplatin toxicity; oxidative density and the foundation of ROS lead to a spreading out in the release of pro-inflammatory cytokines, for example, tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) These later agents hasten the apoptotic process and cellular injury. ROS are potent oxidizing and reducing agents that result in cell membrane damage by activating neutrophils and lipid peroxidation (figure 2.5) (Kwak et al., 2007, Chang et al., 2007b).

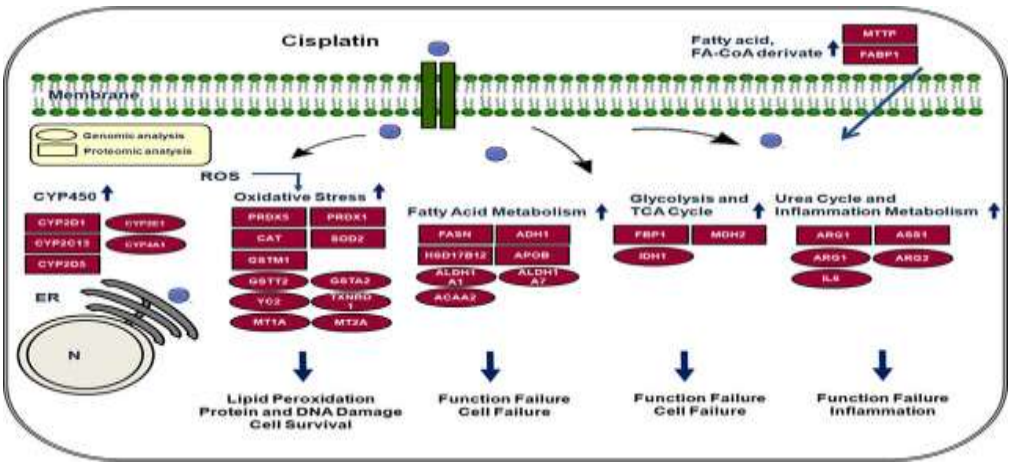

Figure 2.5: Shows model of cisplatin hepatoxicity (Cho et al., 2012)

3. Neurotoxicity

Cisplatin treatment caused a dose-subordinate increment in apoptotic neuronal cells death, it led to increased reactive oxygen species production and DNA damage (Alias et al., 2011, Agarwal et al., 2008b, Buege and Aust, 1978).

Cisplatin usage leads to unbearable neuropathic indications, stopping their administration at the actual dosages and period. tactile nerve filaments give off an impression of being the most influenced part by cisplatin, prompting symmetrical glove and stocking sort of tangible misfortune, deafness, shivering, tingling, pain, and burning sensation. Some of these symptoms may persist for a long period of time and may be complicated even after treatment cessation (figure 2.6.) (Agarwal et al., 2008b, Alkondon et al., 2009).

Some clinical and hereditary highlights of patients may assume play role and make them progressively defenseless to creating serious neurotoxicity during treatment with cisplatin. In a recent study found that male patients experiencing more severe acute neuropathic symptoms than female patients (Buege and Aust, 1978, Volbracht et al., 2006).

Neurotoxicity, as a rule, described by peripheral neuropathies. The neuropathies generally occur after cisplatin therapy however, neurologic manifestations occur after a single dose, neurologic examination should also be performed regularly (Volbracht et al., 2006, Chtourou et al., 2015, Belzung and Griebel, 2001, Begley and Ellis, 2012).

Cisplatin-induced neurotoxicity occurs due to increased oxidative stress, proinflammatory cytokines, mitochondrial dysfunction, DNA damage and apoptotic cell death resulting in various morphological changes in the neurons such as axonal shrinkage and demyelination, Several recent pharmacogenomics studies have proposed that patients with deficiency of glutathione transferases genes are more supposed to develop neuropathy during cisplatin treatment due to decreased drug detoxification (Alkondon et al., 2009, Bardgett et al., 2003, Buege and Aust, 1978).

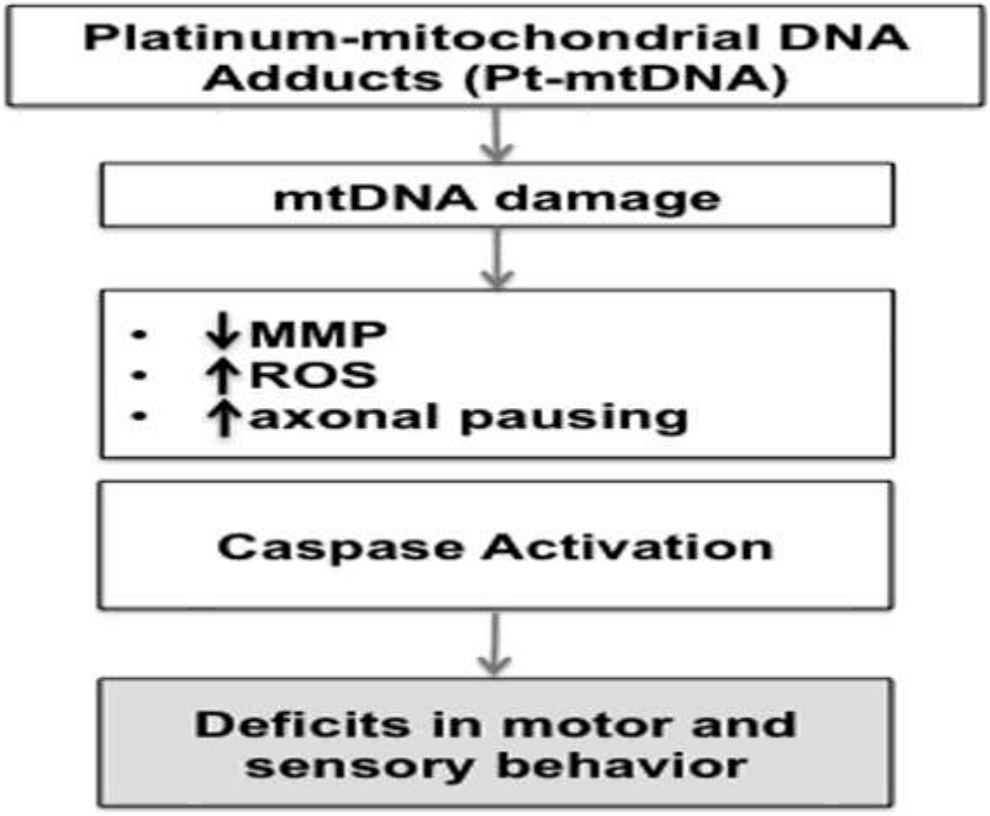

Figure 2.6: Shows mechanism of cisplatin induced neurotoxicity (Podratz et al., 2017).

4. Minor Adverse Reactions

Hematologic myelosuppression happens in 25% to 30% of patients treated by cisplatin. Anemia and thrombocytopenia are increasingly seen at higher dosage (>50 mg/m2). Fever, nausea, vomiting, urination, diarrhea, and infection have

additionally been accounted in patients treated with cisplatin (Pu et al., 2001, Stewart and Bolt, 2012, Belzung and Griebel, 2001).

Ototoxicity of cisplatin is cumulative, audiometric testing ought to be performed preceding starting treatment and before each consequent portion of medication (Hill et al., 2008).

It has been recommended that serum electrolyte disturbances (hypomagnesemia, hypocalcemia, hyponatremia, hypokalemia, and hypophosphatemia) developing concurrently with cisplatin treatment, have been accounted to occur in patients treated with cisplatin and are probably related to renal tubular damage (Chang et al., 2007a). Tetany has been accounted for in those patients with hypocalcemia and hypomagnesemia. Generally, ordinary serum electrolyte levels are reestablished by regulating supplemental electrolytes. Hyperuricemia has been accounted for happening at approximately the same frequency as the increments in BUN and serum creatinine (Devarajan et al., 2004, Kounis et al., 2016).

Cisplatin is teratogenic and embryotoxic and leads to fetal damage is directed to a pregnant patient, also it is mutagenic in bacteria and produces chromosome aberrations in animal cells in tissue culture (Reissig et al., 2009, Reyner and Horne, 2002).

2.2.4. Contraindication

Cisplatin is banned in patients with marked hypersensitive responses to platinum-containing mixes. Cisplatin usually produce a cumulative nephrotoxic

effect which is potentiated by other nephrotoxic agents such as aminoglycoside antibiotics. The Scr, BUN, magnesium, sodium, potassium, and calcium levels ought to be estimated before starting treatment, and preceding each consequent course, care ought to be occupied in dose selection, and renal function should be monitored (Rogawski and Wenk, 2003, Pabla and Dong, 2008).

There are reports of severe neuropathies in patients using higher doses and frequencies of cisplatin. It may be irretrievable and marked as paresthesias, areflexia, vibratory sensation and loss of motor function has been reported widely in aged patients peripheral neuropathy. Anaphylactic-like reactions have also been reported (Dziewczapolski et al., 2009).

Hepatotoxicity showed higher incidences in the elderly compared with younger patients (Ciarimboli et al., 2005, Hill et al., 2008).

2.3. Memantine Hydrochloride

Memantine is uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist with strong voltage-dependency (Unger et al., 2006). The chemical name of memantine is 1-amino-3,5-dimethyladamantane hydrochloride. The molecular formula is C12H21N•HCl and the molecular weight is 215.76. Memantine is soluble in water and occurs as a fine white to off-white powder (figure 2.7) (Unger et al., 2006, Verkhratsky and Kirchhoff, 2007). Memantine is accessible for the oral administration as capsule-shaped, film-coated tablets. The tablets likewise contain the accompanying dormant fixings: microcrystalline cellulose/colloidal silicon

dioxide, powder, croscarmellose sodium, and magnesium stearate (Pabla and Dong, 2008, Verkhratsky and Kirchhoff, 2007, Naqshbandi et al., 2012, Malik et al., 2006).

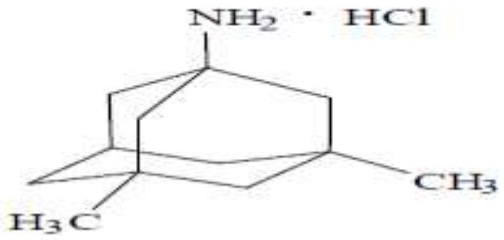

Figure 2.7:- Shows the Chemical structure of memantine(Kornhuber et al., 2007).

2.3.1. Mechanism of action

Memantine is proposed to apply its helpful impact through its activity as a low to direct affinity uncompetitive NMDA receptor enemy, which ties especially to the NMDA receptor-worked cation channels. It antagonizes the special effects of pathologically elevated levels of glutamate that may result in neuronal dysfunction (Muggia, 2009, Verkhratsky and Kirchhoff, 2007).

Glutamate helps to send messages between nerve cells. Memantine protects brain cells by blocking the impacts of excess glutamate (Merouani et al., 1997, Motamedi et al., 2014). Glutamate is discharged in unreasonable sums when neuronal cells are harmed, this makes the cerebrum cells be harmed further (Zhang et al., 2004a, Muggia, 2009, Pabla and Dong, 2008, Lee, 2003).

2.3.2. Pharmacokinetics

Memantine is completely absorbed by oral route and the bioavailability is almost 75%. Time to greatest plasma fixation following single oral portion of 10 mg memantine went between 3 to 7 hours. It has a terminal elimination half-life of about 80-100 hours, with the majority of the dose excreted unchanged in urine (Mohammad et al., 2007b, Morisaki et al., 2008). There is no indication that the presence of food influences the absorption of memantine. Memantine has undeviating pharmacokinetics by the useful therapeutic dose and the plasma protein binding is around 45% and quickly crosses the blood-brain barrier (Militante and Lombardini, 2002, McKeage et al., 2001). Memantine undergoes little metabolism, excreted unchanged in urine (75-90%). The rest of the dose is changed over principally to three polar metabolites: the N-gludantan conjugate, 6-hydroxy memantine, and 1-nitroso-deaminated memantine. These metabolites possess minimal NMDA receptor antagonist activity (Morisaki et al., 2008, Agarwal et al., 2008b). The absolute renal clearance is accomplished by tubular secretion, renal disposal rate of memantine under alkaline urine conditions might be diminished by a factor of 7 to 9 resulting in expanded increased plasma levels of memantine (Naqshbandi et al., 2012, Mohan et al., 2006, Moore et al., 2006).

2.3.3. Pharmacodynamics

Pharmacodynamics indicated low to insignificant partiality for GABA, benzodiazepine, dopamine, adrenergic, histamine, and glycine receptors and for voltage-subordinate Ca2+, Na+ or K+ channels. Memantine likewise demonstrated hostile impacts at the 5HT3 receptor with strength like that for the NMDA receptor and

blocked nicotinic acetylcholine receptors (Kart et al., 2010, Kelland, 2007, Kim et al., 2004).

1. Reduced hepatic function

Memantine is processed into a metabolite with no NMDA-antagonistic activity and is discharged primarily in an unchanged structure by the kidneys. In a study of the pharmacokinetics of memantine in a patient with moderate hepatic impairment, it didn't altogether change the pharmacokinetics of memantine following the administration of a solitary 20 mg oral dose of memantine (Chipana et al., 2008, Ghaima et al., 2013).

2. Reduced renal function

In a study of normal and reduced renal function (serum creatinine result demonstrated a critical increment following a solitary 20 mg oral portion of memantine, was 14% and 39% respectively (Aracava et al., 2005, Arendash et al., 2004).

2.3.4. Non-clinical toxicology: Carcinogenesis, Mutagenesis, and Impairment of Fertility

There was no confirmation of carcinogenicity in a study on mice at doses up to 20 mg/kg/day for 8 weeks (Lee et al., 2007, Vladimirov and Proskurnina, 2009, Weinstein et al., 2000a). Memantine not produced any signs of genotoxic effect when evaluated in a sequence of testes as; an in vitro E. coli turn around transformation test, an in vitro chromosomal distortion test in human lymphocytes, an in vivo cytogenetics

examine for chromosome harm in rodents, and an in vivo mouse micronucleus measure. No impairment of fertility or reproductive performance was seen in rats treated by 18 mg/kg/day orally (Yuede et al., 2007, Zadak et al., 2009, Xia et al., 2009, Yin et al., 2017).

2.3.5. Neurobehavioral and pharmacological basis of memantine treatment

Memantine's mechanism of action in neurological disorder is a voltage-dependent, low-moderate attraction, uncompetitive NMDA receptor opposition with fast-blocking/unblocking kinetics (Unger et al., 2006, Umeda et al., 2004).

The quick on/off kinetics is also vital as it allows memantine to stop pathologic initiation of the glutamate receptors. Memantine blocks the effects of irregular glutamate activity that may lead to neuronal cell injury and cognitive dysfunction. Like other NMDA receptor antagonists, memantine clinically applicable concentrations memantine can encourage synaptic plasticity and preserve or enhance memory in animal models of AD. In addition, memantine can protect against the excitotoxic destruction of cholinergic neurons (van der Worp et al., 2010, Ozkan et al., 2015, Mohammad et al., 2008, Tozzi et al., 2007).

2.4. Immunohistochemistry (IHC)

The immunohistochemistry (IHC) is a mixture of immunological and biochemical responses imagined with a photonic microscope, also refers to as the procedure of restricting proteins compartments of tissue section take advantage of the

principle of antibodies attaching precisely to antigens in living tissues. It takes its name from "immune," in reference to antibodies used in the procedure and "history" meaning tissue (Merouani et al., 1997, Ramos et al., 2005, Morotti et al., 2006).

IHC is a key tool for the investigation and localization of objective particles within tissues. It is used regularly for virtually every feature of contemporary biomedical study. Technical ease of use, rapidity, and reliability usually determine the techniques utilized in academic or medical settings. (Ramos et al., 2005, Morotti et al., 2006, Yang et al., 2013, Yin et al., 2017).

The immunohistochemical procedure has fortified the histopathologist to tackle the most common analytical complications in cancer pathology particularly the characterization of the undifferentiated or poorly differentiated malignant tumors. No other method, during the past fifty years, has had such a main impact on histopathology (Ramos et al., 2005, Wei et al., 2006, Wang et al., 2017, Yang et al., 2013).

IHC is the study of antigen to antibody interactions and how these reactions are visualized in tissues. The crucial antibody is subjected to the tissue, where the antigen is (or is suspected to be) present. The antigen is prepared from a mixture of several proteins in a specific sequence and conformation. The site on the antigen where the antibody binds is referred to as the “epitope” and is consist of generally 5-16 amino acids, which may represent a small percentage of the length of the total antigen. Single-antigen may have numerous “epitopes” (antibody binding sites). Each binding site is given a different name, referred to the antibody clone name. The antibody will bind to the epitope and the detection system used will allow for

visualization of this antibody-antigen reaction (Ramos et al., 2005, Savic et al., 2008, Morotti et al., 2006, Yin et al., 2017).

IHC provides the greatest straight method for recognizing both the cellular and subcellular spreading of protein and can provide a relatively quick sign of gene manifestation or protein distribution (Morotti et al., 2006, Moore, 2006, Yang et al., 2013).

2.4.1. IHC methods

An antigen-antibody interaction can be visualized using the following methods:

1. Direct method

Is a one-step staining method and involves a labeled antibody (Diverse tags have been used, including fluorochromes, enzymes, colloidal gold and biotin) rejoining in a straight line with the antigen in tissue segments. While this method exploits only single antibody and therefore is simple and quick, the sensitivity is lower due to little signal extension and is less frequently used than indirect methods (Levin, 2004, Nagane et al., 2001, Ramos et al., 2005, Yang et al., 2013, Yin et al., 2017).

2. Indirect method

Comprises an unlabeled primary antibody that fixes to the target antigen and a categorized secondary antibody that counters with the primary antibody. The

secondary antibody must be raised against the IgG of the animal species in which the primary antibody has been elevated. This method is extra sensitive than direct detection plans because of signal strengthening; due to the binding of several secondary antibodies to each primary antibody if the secondary antibody is conjugated to the fluorescent or enzyme receptor (Ramos et al., 2005, Yang et al., 2013, Yin et al., 2017).

3. Avidin-biotin complex (ABC) method

ABC method is typical IHC technique and broadly used method for immunohistochemical stain. Avidin is a great glycoprotein, which can be labeled with peroxidase or fluorescent and has a high attraction for biotin. The technique involves three layers. The first layer is the unlabeled primary antibody. The second layer is the biotinylated secondary antibody. The third layer is a complex of avidin-biotin peroxidase-linked with the appropriate label. The peroxidase is then developed by the DAB or another substrate to produce different colorimetric end products (Ramos et al., 2005, Yang et al., 2013, Yin et al., 2017).

4. Labeled avidin-biotin (LAB) or labeled streptavidin-biotin (LSAB) method

Streptavidin, consequent from *Streptococcus avidini*, is a modern revolution for the exchange of avidin. LSAB is precisely comparable to typical ABC method. The first layer is the unlabeled primary antibody. The second layer is the biotinylated secondary antibody. The third layer is the enzyme-streptavidin conjugates (HRP-

Streptavidin or AP-Streptavidin) to replace the complex of avidin-biotin-peroxidase. A current report recommends that the LSAB method is about 5-10 times more sensitive than the standard ABC method (Ramos et al., 2005, Yang et al., 2013, Yin et al., 2017).

5. Peroxidase–anti-peroxidase (PAP) method

PAP technique is an additional expansion of the indirect technique and it includes a third layer which is a rabbit antibody to peroxidase, attached with peroxidase to make a steady peroxidase anti-peroxidase compound. The sensitivity is about 100-1000 times greater meanwhile the peroxidase particle is not chemically conjugated to the anti IgG but immunologically bound and misses nothing of its enzyme activity (Ford et al., 2005, Frezza et al., 2010, Ramos et al., 2005).

6. Polymeric methods

Polymeric Procedures are grounded on dextran polymer expertise and original technique of polymerizing enzymes and binding these polymers to antibody (Inao et al., 2012, Jiang et al., 2006, Ramos et al., 2005, Yin et al., 2017).

2.4.2. Applications of IHC

1. Diagnosis of tumors of uncertain histogenesis.

2. To identify abnormal protein deposits within cells.

3. IHC is broadly used in the elementary investigation to realize the distribution and localization of biomarkers and differentially articulated proteins in diverse portions of biological tissue.

4. IHC is a highly sensitive and specific method, particularly advantageous as an analytic tool for transferable illnesses in animals.

5. Categorization of leukemia and lymphomas.

6. Identifying the origin and type of secondary deposits.

7. Identification of hormone receptors, which are of prognostic value as estrogen, progesterone (Ramos et al., 2005, Yang et al., 2013, Yin et al., 2017).

2.5. Free radicals

these can be well-defined as particles enclosing unpaired electrons, which typically confers a substantial amount of reactivity as a free radical (Zadak et al., 2009). Those radicals derived from oxygen represent the most important class generated in living systems. In addition, free radicals recruit autocatalytic responses; elements that react with free radicals are in turn transformed into free radicals, further broadcasting the damage chain (Zadak et al., 2009, Yohay et al., 2014, Valko et al., 2004).

Reactive oxygen species (ROS) contains a number of active metabolites including hydroxyl radical (OH・), superoxide anion (O2・-) and peroxyl radical and hydrogen peroxide (H2O2). Reactive nitrogen species (RNS), include nitric

oxide (NO) and nitric dioxide (NO2) (Valko et al., 2004, Chang and Liu, 2007, Choi et al., 2004, Droge, 2002).

2.5.1. ROS and RNS

(i) Generated during irradiation by UV light, by X-rays, and by γ-rays.

(ii) Products of metal-catalyzed reactions.

(iii) Existing as contaminants in the atmosphere.

(iv) Formed by neutrophils and macrophages during inflammation.

(v) By-products of mitochondria-catalyzed electron transport reactions (Valko et al., 2004, Cheema et al., 2004, Choi et al., 2004).

2.5.2. Terminology of free radicals

The associated expressions of oxidative stress, oxidative damage, free radical, and antioxidant have developed a combined fragment of the scientific terminology and are regularly used in a diversity of scientific considerations and topics by researchers (Pelle et al., 2003). Free radicals, identified since the foundation of the 20th century, were originally used to designate transitional mixtures in organic and inorganic chemistry, and numerous descriptions for them were recommended; in 1954 were these radicals suggested as vital performers in living surroundings and accountable for harmful progressions in the cell (Valko et al., 2004, Droge, 2002).

These radicals are well-thought-out as chief players in biological responses, cellular response, and clinical outcome (Droge, 2002). Chemically, each compound, containing oxygen that can receive electrons is an oxidant or oxidizing agent; in

contrast, an element that gives electrons is a reducing agent (Valko et al., 2004, Droge, 2002, Agarwal et al., 2006, Ali et al., 2008a). The radical group contains complexes such as nitric oxide radical (NO•), superoxide ion radical (O•2), hydroxyl radical (OH•), peroxyl(ROO•) and alkoxyl radicals (RO•), and one form of singlet oxygen (1O2) (Valko et al., 2004, Droge, 2002, Dixon et al., 2008).

The occurrence of one unpaired electron results in high reactivity of these species by their affinity to donate or obtain another electron to attain stability (Droge, 2002, Egashira and Takayama, 2002). Here is a cluster of non-radical complexes that insurances a large variation of constituents, some of which are extremely reactive although not radical by definition; among these compounds produced in high concentrations in the living cell are hypochlorous acid (HOCl), hydrogen peroxide (H2O2), organic peroxides, aldehydes, ozone (O3), and O2 (Egashira and Takayama, 2002, Ali et al., 2008a, Blumenthal et al., 2000).

Antioxidant (reducing agent) can be classified as a compound capable of preventing the peroxidation process, or biological oxidative damage (Egashira and Takayama, 2002, Dixon et al., 2008). Normally body must control the presence status of each of pro-oxidants and antioxidants constantly; this equilibrium must be firmly controlled and tremendously significant for conserving biological, cellular and biochemical functions (Valko et al., 2004, Dixon et al., 2008) any interference in this balance in any direction might be deleterious for the body (Dixon et al., 2008, Droge, 2002). Fluctuating the equilibrium in the direction of intensification in the prooxidant over the measurements of the antioxidant is demarcated as oxidative stress and oxidative damage (Droge, 2002, Fearon et al., 2013, Valko et al., 2004, Galanski, 2006, Devi et al., 2005).

2.5.3. Chemical Properties of ROS

ROS are short-lived species, react quickly with other molecules; its reaction rate constants for biological components are extremely high. The life span of other radicals is also short but depends on the environmental medium (Valko et al., 2004, Devi et al., 2005, Dickey et al., 2004).

Toxicity is correlated with reactivity; the longer half-life of a species implies higher toxicity of the compound by allowing it adequate time to diffuse and reach a sensitive location where it can interact and cause damage a long distance from its site of production (Valko et al., 2004, Ali et al., 2008a, Al-Kahtani et al., 2014). For example, the relatively long half-life of superoxide radicals can be produced in the mitochondrial membrane, diffuse towards the mitochondrial genome, and reduce transition metals bound to the genome. The extraordinary reactivity of radicals demonstrates the probable poisonous effect and complications in avoiding oxidative damage. To avoid the communication among radicals and biological targets, the antioxidants must be existing at the site where the radicals are being formed in a direction to compete with the radical for the biological substrate (Valko et al., 2004, Amin and Hamza, 2006, Barbosa et al., 2008, Blumenthal et al., 2000).

2.5.4. Chemical Qualities and Reactivities of ROS

1. Superoxide Anion Radical (O•2/HO•2)

superoxide can happen in the form of either O•2 or, at low pH, hydroperoxyl (HO•2). The latter can simply infiltrate living membranes than the charged form. Hydroperoxyl radical can, therefore, be considered as an important species. In a

hydrophilic environment both the O•2 and HO•2 can act as reducing agents capable of reducing ferric (Fe+3) ions to ferrous (Fe+2) ions; inorganic solvents the solubility of O•2 is higher, and its ability to act as a reducing agent is increased, capable of attacking positively charged centers (Valko et al., 2004, Blumenthal et al., 2000).

2. Hydroxyl Radical (OH•)

The hydroxyl radicals have extremely high reactivity, they are short-lived radicals with high affinity toward other molecules. It is a dominant reacting manager that responds at a high rate with other organic and inorganic molecules in the cell, including DNA, proteins, lipids, amino acids, sugars, and metals; it is considered the most reactive radical in biological systems (Blumenthal et al., 2000, Bhandari et al., 2008).

3. Hydrogen Peroxide (H2O2)

H2O2 molecules are considered reactive oxygen metabolites, they can cause damage to the cell at a low concentration. They can easily penetrate biological membranes. Their harmful biochemical properties can be separated into two classes of direct activity and indirect activity, creating from their reacting properties, in which they assist as a basis for more poisonous type, such as OH• or HOCl. Direct activities of H2O2 include degradation of haem proteins; release of iron; inactivation of enzymes (Bhandari et al., 2008, Blumenthal et al., 2000, Chen et al., 2013).

4. Nitric Oxide (NO•) and Peroxynitrite (ONOO–)

The maximum significant reactions have been seen in physiological conditions is that of superoxide and nitric oxide radicals resulting in peroxynitrite; this reaction helps to maintain the balance of superoxide radicals and other ROS and is also important in redox regulation (Egashira and Takayama, 2002, Chen et al., 2013). The protonated formula of peroxynitrite (ONOOH) is a commanding oxidizing mediator that cause exhaustion of sulfhydryl (–SH) groups and oxidation of many molecules producing damage (Bhandari et al., 2008, Blumenthal et al., 2000).

2.5.5. Roles of free radicals

ROS and RNS may play a dual role in biological systems, can be either harmful or beneficial. The beneficial effects of ROS seen at low to moderate concentrations and comprise biological characters in cellular reactions to noxious, in defense against infectious agents and induction of a mitogenic response (Blumenthal et al., 2000, Boulikas and Vougiouka, 2004, Chang and Kim, 2007). The destructive consequence of free radicals triggering possible biological impairment and oxidative stress, it occurs when this balance is disrupted due to depletion of antioxidants or excess accumulation of ROS, or both (Cai et al., 2018, Chang et al., 2007a).

The equilibrium among advantageous and damaging properties of free radicals is a very significant trend of living creatures and is attained by mechanisms called redox regulation, it defends living organisms from oxidative stresses and

preserves redox homeostasis by controlling the redox status (Chakravarthi et al., 2006, Chang and Kim, 2007, Chen et al., 2013).

2.5.6. Sources of ROS

The living compartment is constantly showing a large variety of ROS and RNS from both exogenous and endogenous sources. Exposure of living organisms to ionizing and nonionizing irradiation constitutes a major exogenous source of ROS, such as hemolytic cleavage of H2O2 by UV radiation which yields OH• radicals (Cheeseman, 1993, Chen et al., 2008a, Chang et al., 2007b). Certain types of drugs and/or their metabolites are considered as a major source of ROS (Scandalios, 2005). There are drugs, such as bleomycin and doxorubicin, where their mechanisms of action as cytotoxic agents are mediated via production of ROS; those like nitroglycerine and other nitrates are NO • donors, and some have the ability to produce ROS indirectly. Moreover, some narcotic agents and anesthetic gases are considered major contributors to the production of ROS in the biological system (Chang and Kim, 2007, Chanvorachote et al., 2006, Cheeseman, 1993, Chen et al., 2013).

2.5.7. Free Radicals as a Cause of Oxidative Damage

Reactive oxygen species (ROS) and other free radicals are highly reactive, prone to cause damage, and they are potentially toxic, mutagenic, or carcinogenic (Chakravarthi et al., 2006, Chang et al., 2007a, Cheeseman, 1993). The objectives for ROS damage include all major groups of biomolecules, summarized as follows:-

1. Effects on Nucleic Acids

Free radicals, especially reactive oxygen species (ROS), have been presented to be mutagenic, an outcome that ought to be imitative of biochemical alteration of DNA (Chang and Kim, 2007, Chang et al., 2007a). A number of variations (cleavage of DNA, DNA-protein cross relations, oxidation of purines) are due to responses with ROS, especially OH•; if the DNA repair systems are not able to immediately regenerate intact DNA, a mutation will result from incorrect base pairing during replication (Chang and Kim, 2007, Chan et al., 2000, Chang et al., 2007a).

This appliance may partially elucidate the great commonness of cancer in individuals exposed to oxidative stress. The feature that apoptosis in some cases is facilitated by ROS may in part be due to ROS-derived harm to DNA, but is also related to augmented mitochondrial permeability, released cytochrome C, increased intracellular Ca2+, and other effects (Chang et al., 2007a, Chanvorachote et al., 2006, Chang and Kim, 2007).

2. Effects on Lipids

Lipid peroxidation is possibly the most discovered part of the investigation when it comes to ROS. Polyunsaturated fatty acids because of their numerous paired bonds, are brilliant goals for free radical outbreaks; such oxidation is also vital for the cohort of atherosclerotic plaques (Chan et al., 2000, Slimen et al., 2014). The mechanism for plaque formation contains oxidation of little thickness lipoproteins (LDL), uptake of those particles by phagocytes in the subendothelial space via their hunter receptor, and finally, accumulation of these phagocytic cells in the sub-

endothelial space, where they stimulate the formation of atherosclerotic plaques (Chen et al., 2013, Hung et al., 2010). Circulatory sickness with plaque development establishes a large part of the total burden of disease; therefore, prevention or decrease of lipid peroxidation is of significant medical importance (Cheeseman, 1993, Chang and Liu, 2007).

3. Effects on Proteins

Proteins can serve as targets to be attacked by ROS, which must be shown to counter with several amino acid residues, generating less active enzymes and denatured nonfunctioning proteins. Among the most susceptible amino acids are sulfur- (or selenium)-containing residues (Chanvorachote et al., 2006, Chen et al., 2013). Proteins can go through direct and indirect damage succeeding interaction with ROS, including peroxidation, damage to specific amino-acid residues, changes in their tertiary structure, degradation, and fragmentation. The consequences of protein damage as a response to stress including loss of enzymatic activity, altered cellular functions such as energy production and changes in the type and level of cellular proteins (Chang et al., 2007b, Chen et al., 2013).

2.5.8. Relation between oxidative stress and carcinogenesis

ROS are involved in carcinogenesis through possible mechanisms including effects on signal transduction, transcription factors and gene mutations (Cheema et al., 2004, Chen et al., 2008a, Chen et al., 2008b).

1. Lipid peroxidation

Double bonds in membrane polyunsaturated fatty acids are vulnerable to attack by ROS. The hydroxyl radical removes a hydrogen atom from the unsaturated lipids of the membrane, a process that forms a free lipid radical, that in turn, reacts with molecular oxygen and forms lipid peroxide radical (Chakravarthi et al., 2006, Vareed et al., 2007). Later this can function as an initiator, removing another hydrogen atom from a second unsaturated lipid. A lipid peroxide and a new lipid radical result and an abnormal chain reaction is started. Lipid peroxides are unstable and break down into smaller molecules and results in a loss of membrane integrity (Circu and Aw, 2010, Cheuk et al., 2009).

2. Protein interactions

Hydroxyl radicals attack proteins. The sulfur-containing amino acids are especially vulnerable to attack by OH·. As a result of oxidative damage, proteins undergo fragmentation, cross-linking, aggregation and degradation. ROS have been implicated as the second messenger in regulating gene expression. It has been shown that oxidative stress can modulate the activity of protein kinases, which in turn phosphorylate a wide range of cellular proteins (Chakravarthi et al., 2006, Circu and Aw, 2010, De et al., 2000).

3. DNA damage

The hydroxyl radical reacts with all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone, also cause a variety of structural alterations to include strand breaks, modified bases, and cross-links between strands Oxidized and injured DNA have the potential to induce genetic mutation (Chan et al., 2009a, Cheema et al., 2004, Cheeseman, 1993).

4. Metabolism of free radicals

ROS are a natural part of metabolism. They are constantly generated in the body and are rapidly removed by enzymatic and non-enzymatic antioxidants. Antioxidant molecules interact with ROS to either eliminate or to minimize their effects; this maintains the pro-oxidant/antioxidant balance (Yeung et al., 2007, Mabrouk et al., 2010, Dixon et al., 2008).

The excess of free radicals results in a series of events mediating a progressive deterioration of a cellular structure and function and this can lead to the differentiation of neoplastic tissues. It has been reported that ROS/RNS induce various types of oxidative DNA lesions that are thought to be important in carcinogenesis (Cheema et al., 2004, Amin and Hamza, 2006, Ali et al., 2008b, Agarwal et al., 2006).

2.6. Antioxidants

The antioxidant is capable of inhibiting oxidation of other molecules, exposure to free radicals has led to developing a series of defense mechanisms in the body in order to prevent and repair the altered status induced by free radicals through the initiation of physical and antioxidant defenses (Circu and Aw, 2010, Agarwal et al., 2006). Antioxidants neutralize free radicals and play an important role in maintaining the health and integrity of the cells. The balance between free radicals and antioxidants is thought to be strongly related to the good health status of the body (Ali and Al Moundhri, 2006, Apak et al., 2007, Bachowski et al., 1998).

A variety of enzymes synthesized in the body and function as antioxidants; such as enzymatic antioxidant defenses which includes superoxide dismutase, glutathione peroxidase, glutathione transferase, and catalase. Non-enzymatic antioxidants are represented by ascorbic acid, tocopherol, glutathione, and other antioxidants. Normally, there is a balance between activities and the levels of these antioxidants. There is a link between increased levels of ROS and disturbed activities of enzymatic and non-enzymatic antioxidants in cells and tissues (Circu and Aw, 2010, Amin and Hamza, 2006, Barbosa et al., 2008).

A good antioxidant should able to:-

1. Quench free radicals.

2. Chelate redox metals.

3. Have a positive effect on gene expression.

4. Be readily absorbed.

5. Have enough concentration in cells and tissues at a physiologically considerable level.

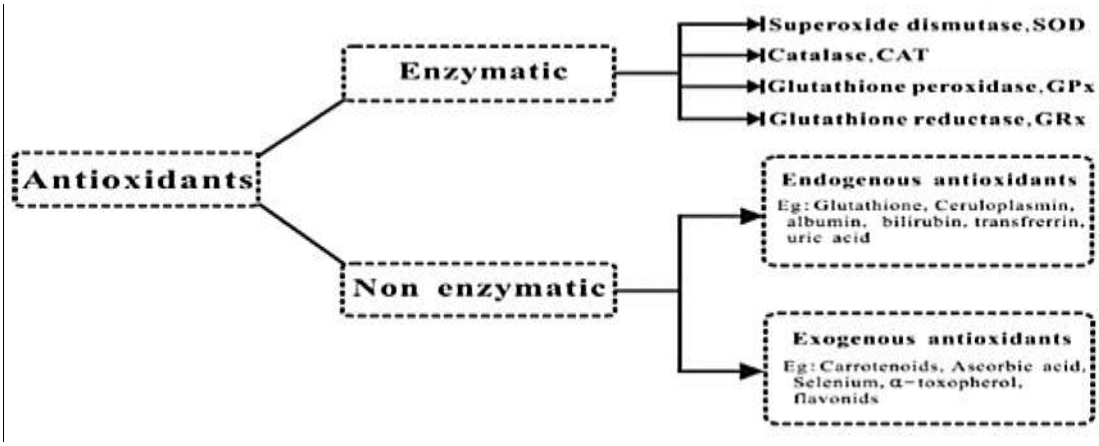

Figure 2.8: Enzymatic and non-enzymatic kinds of antioxidants (Li et al., 2016)

2.7. Nicotinic Acetylcholine Receptors (nAChRs)

Nicotinic Acetylcholine Receptors (nAChRs) are ligand-gated ion channels, these receptors are proteins respond to the excitatory neurotransmitter like acetylcholine, it is a non-selective cation channel, several different positively charged ions $Ca^{2+,}$ Na^{+} and K^{+} can cross through It (Wu et al., 2010). nAChRs are abundant in the central and peripheral nervous system, muscle, and many other tissues, they are the primary site in muscle for communicating motor nerve and muscle in order to control muscle contraction (Zhu et al., 2014, Yonezawa et al., 2006).

In the peripheral nervous system:

(1) They are responsible for transmitting signals from presynaptic to the postsynaptic cells within the sympathetic and parasympathetic nervous system (Wu et al., 2010).

(2) these receptors are found on skeletal muscle and receive acetylcholine that released and signal for muscular contraction (Aras et al., 2015, Aracava et al., 2005, Zhao et al., 2001).

Nicotinic acetylcholine receptor subunits consist of an extracellular domain (ECD), four α-helical transmembrane domains, and an intracellular loop between the M3 and M4 transmembrane domains. When agonists such as acetylcholine and nicotine binds, the pore opens and cations flow according to their electrochemical gradient (Zhang et al., 2007, Yohay et al., 2014).

nAChRs present in muscle is composed of α, β, γ and δ subunits. Neuronal nAChRs are composed of α2-α11 and β2-β4 subunits. The neuronal α7 receptor subtype is one of the most abundant nAChRs in the central and peripheral nervous system. This subunit stoichiometry is important in determining its pharmacology, stability, and subcellular location; these properties contribute to the development of disease or dependence states (Bardgett et al., 2003, Aracava et al., 2005, Aras et al., 2015).

The activation of α7 nAChRs explains the state of neurons through two main mechanisms. Firstly, the movement of cations causes a depolarization of the plasma membrane which leads to an excitatory postsynaptic potential in neurons, secondly by the activation of voltage-gated ion channels (Wu et al., 2010).

Prolonged exposure to a stimulus often results in decreased responsiveness of that receptor toward a stimulus, termed receptor desensitization (Yang et al., 2013, Yohay et al., 2014, Wu et al., 2010).

2.8. Myeloperoxidase (MPO)

Myeloperoxidase (MPO) is a major protein of neutrophils, the most abundant leukocyte in the body (Yokoo et al., 2009). It catalyzes the formation of powerful reactive oxidants which can have profound biological effects by killing microbes, but also causing host tissue injury, as has been demonstrated in many inflammatory diseases (Zhu et al., 2014). MPO affects tissue inflammation and damage in various models of glomerulonephritis (kidney inflammation) and rheumatoid arthritis (joint inflammation). Acute kidney injury (AKI), resulting from different factors including toxic effects of drugs and various infections, is one of the major causes of morbidity and mortality in patients. Inflammation, mediated by kidney-infiltrating leukocytes such as neutrophils plays a key role in the pathogenesis of AKI (Zhu et al., 2014, Yokoo et al., 2009, Yao et al., 2007).

AKI can be induced by a single injection of the chemotherapeutic agent, cisplatin is used in cancer patients but causes renal damage via direct effects on kidney cells and subsequent accumulation of injurious leukocytes (Yokoo et al., 2009). Neutrophils and MPO are present in kidneys of animals treated with cisplatin-induced AKI, suggesting that MPO has the potential to contribute to renal damage in this model (Zhang et al., 2010, Abdel Moneim, 2014).

2.9. Acetylcholine esterase (ACHE)

Cholinergic neurons are involved in many functions, including attention, learning, memory, cognition, control of sleep, motor function. Dysfunctions of central

cholinergic systems, involved in a variety of neuropsychiatric disorders (Akman et al., 2015, Ali et al., 2008b, Aracava et al., 2005, Cirrito et al., 2005).

Degeneration of basal neurons reduces the cholinergic level in the cortex and hippocampus and strongly responsible for cognitive dysfunction. Acetylcholine (ACh) is inactivated by acetylcholinesterase which is responsible for the termination of cholinergic transmission by cleavage of ACh to acetate and choline. Inhibition of AChE by cholinesterase inhibitors causes an increase in ACh levels. Treatment of neuropsychiatric disorders with cholinesterase inhibitors causes symptomatic treatment and seems to stop disease progression (Alias et al., 2011, Aracava et al., 2005, Aras et al., 2015, Sinko et al., 2007).

AChE has a high catalytic activity, each molecule has the ability to degrade about 25000 molecules of acetylcholine per second, it contains two active sites; the anionic site and the esteratic site (Cheema et al., 2004, Cirrito et al., 2005).

The anionic site modifies the positive quaternary amine of acetylcholine, cationic substrates, and inhibitors. The cationic substrates are bound by the interaction of 14 aromatic residues to the active site. Among the aromatic amino acids, tryptophan results in a decrease in the reactivity (Behrens et al., 2000, Boyer et al., 2006).

The esteratic site, where acetylcholine is hydrolyzed to acetate and choline, contains the catalytic subsite of three amino acids: serine, histidine, and glutamate. The hydrolysis of the carboxyl ester leads to the formation of an acyl-enzyme and free choline, later the acyl-enzyme undergoes nucleophilic attack by a water molecule, assisted by the histidine, liberating acetic acid and regenerating the free enzyme (Aracava et al., 2005, Bian et al., 2001, Behrens et al., 2000, Cirrito et al., 2005).

The process of nerve signal transmission occurs by ACh releasing into the synaptic cleft and binds to post-synaptic ACh receptors, conducting signals from the nerve through the synaptic cleft. This signal will be terminated by AChE, which is located on the post-synaptic neuron. The liberated choline is taken up again and ACh is synthesized by combining with acetyl-CoA through the action of choline acetyltransferase (Zha et al., 2017, Zhang et al., 2010, Rogers et al., 2003, Unwin, 2005, Wu et al., 2010, Sinko et al., 2007).

2.10.Serotonin(5-HT)

5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter, derived from tryptophan, predominate in the gastrointestinal tract, blood platelets, and central nervous system, serotonin modulation at the synapses is the main mechanism of action of many classes of pharmacological antidepressants, it has various functions such as regulation of mood, appetite, sleep and cognitive functions regarding memory and learning, behavior, anxiety and sexual function. Serotonin plays a role in wound healing by acting as a growth factor for some types of cells, and allow blood flow to the brain (Miquel et al., 1990, Mendlewicz et al., 2004).

Serotonin is synthesized from L-tryptophan by a short metabolic pathway consisting of enzymes: tryptophan hydroxylase (TPH), aromatic amino acid decarboxylase (DDC) and the coenzyme pyridoxal phosphate (Kaneda et al., 2000). Serotonin secreted out of tissues into the blood, then actively taken up by blood platelets, which store it, then binding to a clot it will release their content of serotonin, where it can serve as a vasoconstrictor and/or a vasodilator (Kane et al., 2000).

The estimated levels of serotonin in the body are fundamental for the maintenance of the functionality of the central nervous system, and for the regulation of the normal physiological status of the body (Kaneda et al., 2000, Morisaki et al., 2008). In abnormal states regarding high concentrations and pathologic states, the action of serotonin is different, in high concentration it acts as a vasoconstrictor by contracting the endothelial smooth muscle. In physiologic states, serotonin leads to vasodilation which occurs through the serotonin-mediated release of nitric oxide from endothelial cells (Miquel et al., 1990, Mendlewicz et al., 2004). Liver Metabolism occurs by oxidation route via monoamine oxidase, the water-soluble end product of the metabolism is excreted by the kidneys (Kaneda et al., 2000, Mansour et al., 2006).

2.11. Dopamine (DA)

(DA, 3,4-dihydroxyphenethylamine) is synthesized by removing a carboxyl group from its precursor L-DOPA, which is synthesized in the brain and kidneys. In the brain, dopamine functions as a neurotransmitter, involved mainly in motor control, lactation, sexual desire, nausea and in the release of various hormones, also act as the main chemical of pleasure (Chen et al., 2013, Liu et al., 2004b, Liu et al., 2004a).

Several important diseases of the nervous system are associated with dysfunctions of the dopamine system, and the mechanism of action some medications used to treat them work by altering the effects of dopamine.

Dopamine is converted metabolically into an inactive product by a set of enzymes; catechol-O-methyl transferase (COMT), monoamine oxidase (MAO) and aldehyde dehydrogenase (ALDH). The homovanillic acid (HVA) is the end product

with no known biological activity, excreted out of the body by the kidneys (Chen et al., 2004b, Chen et al., 2004a, Liu et al., 2004b, Chou et al., 2005).

Dopamine is susceptible to oxidation by direct reaction with oxygen, yielding quinones and various free radicals as byproducts, oxidation can be increased by the presence of ferric iron or other factors (Chou et al., 2005, Dailly et al., 2004).

Due to extensive localization of dopamine receptor to brain areas and its role in wide range of functions, dopaminergic dysfunction has been implicated in the pathophysiology of many mental disorders and motor disabilities, it involved in many physiological processes, the most studied role involves the effects of dopamine on locomotor activity (Dailly et al., 2004). Multiple evidence indicate that locomotor activity is primarily controlled by D1, D2, and D3 dopamine receptors, decrease in dopamine release results in decreased locomotor activity, D2 dopamine receptors seem to be the predominant type of receptors that are involved in regulating of the synthesis rate of dopamine and their release (Chen et al., 2004c, Chen et al., 2004b, Dailly et al., 2004, Liu et al., 2004a).

2.12. Enzyme-Linked ImmunoSorbent Assays (ELISA)

ELISAs are more sensitive, specific and widely used test than other serological tests, it can be performed with minimum equipment. ELISA principle depend on uses of one sub-type of heterogeneous, solid phase to detect the presence of a substance, usually an antigen, a liquid sample is added onto a stationary solid phase with special binding ability, is followed by multiple liquid reagents that are sequentially added, incubated and washed followed by some optical change by which the quantity of the

analyte is measured. The sensitivity of detection depends on a signal produced during the analytic reactions, the signal is generated by enzymes which are linked to the detection reagents in fixed proportions to allow accurate quantification (Mendlewicz et al., 2004, Engvall and Perlmann, 1971, Kohler and Milstein, 1975).

2.13. Clinical Laboratory Parameters Alterations

The following parameters are usually studied on the basis of their potential clinical significance in manifestation the severity of hepatoxicity, nephrotoxicity and neurobehavioral toxicity: Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Blood urea nitrogen (BUN), Creatine kinase (Ck) and Serum creatinine (Scr) concentrations (Blumenthal et al., 2000, Brave et al., 2006)

1. Blood Urea Nitrogen (BUN)

Urea is a nitrogenous waste product that is formed in the liver as the end product of deamination of amino acids. It is transported in the blood to the kidney where it is excreted in the urine. In other words, high blood urea concentrations are indicative of impaired renal function. States associated with elevated levels of urea in blood are referred to as hyper uremia (Berker et al., 2007, Bennett, 1980, Amin and Hamza, 2006).

2. Alkaline Phosphates (ALP)

This is a hydrolytic enzyme acting optimally at alkaline pH. It exists in blood in a distinct form which originates mainly from bone and liver also from other tissues like kidney and placenta. Pathological increases are largely associated with hepatic and bone diseases (Al-Zubaidy et al., 2011, Arany and Safirstein, 2003).

3. Serum creatinine (Scr)

Creatinine represents end product wasted by the kidneys mainly by glomerular filtration. The concentration of creatinine of healthy individual is nearly constant. Abnormally increased level of serum creatinine values indicates decreased excretion and impaired renal function. The creatinine clearance is a good indication of the glomerular filtration rate (GFR) which allows better detection of kidney diseases and monitoring of renal function (Al-Zubaidy et al., 2011, Begley and Ellis, 2012).

4. Creatine Kinase (Ck)

This is an enzyme which consists of isoenzymes mainly of the muscle (Ck-M) and the brain (Ck-B), is the most widely used serum enzyme determination in neuromuscular diseases of domestic animals. Elevated CK levels are observed in cardiac muscle damages and in skeletal muscle diseases (Bergstrom et al., 1999, Brave et al., 2006). A measurement of CK is used especially in the diagnosis of myocardial infarction and myopathies (Brock et al., 2012, Che and Siu, 2010).

5. Alanine aminotransaminase (ALT)

It is an enzyme released into the serum as a result of tissue injury; therefore the concentration of ALT in the serum increased by acute damage to hepatic cells. Alanine aminotransferase (ALT) is an enzyme which catalyzes the conversion of alanine and α-ketoglutarate to pyruvate and glutamate contributing to cellular nitrogen metabolism and liver gluconeogenesis (Chang et al., 2007b, Chang et al., 2007a).

6. Serum Calcium Determination

Calcium plays an essential role in many cell functions, intracellularly in muscle contraction and glycogen metabolism, extracellularly in bone mineralization, in blood coagulation and in the transmission of nerve impulses (Bertram et al., 2004). Cisplatin treatment induces disturbances of electrolyte homeostasis were characterized by hypomagnesemia and hypocalcemia. Moreover, the effect appears to be specific for divalent cations (Chang et al., 2007b, Chan et al., 2009b, Ciarimboli et al., 2005).

7. Serum Sodium Determination

Sodium (Na+) is one of the most important electrolytes, plays vital roles in the maintenance of normal cell functions such as plasma volume, pH balance or transmission of nerve impulses (Ciarimboli et al., 2005). Hyponatremia can occur in nephrotic syndrome, excessive vomiting, and diarrhea, sodium concentration in clinical settings is determined by the Sodium Assay Kit (Colorimetric) offers a simple, two-step colorimetric assay (Che and Siu, 2010, Carrasco et al., 2007).

8. Serum Potassium Determination

Potassium plays a critical role in many body functions such as the normal functioning of heart muscles, smooth muscle contraction, conducting electric signals between nerves (Ciarimboli et al., 2005). Hypokalemia is a potentially life-threatening imbalance and can cause serious symptoms such as palpitations, psychological effects and a decrease in cognitive performance such as learning (Danysz et al., 2000a, Devi et al., 2005).

3.14. Toxicological Studies

1. Cisplatin: Acute Toxicity

Acute toxicity of cisplatin in rats and mice were showed the following toxicity data (LD_{50}) (table 2.1). Clinical signs of intoxication included severe pain (hunched posture, lethargy, orbital tightening, nose bulge, cheek bulge, and changed ear and whisker). Before death, particular signs are noticeable such as ataxia with loss of coordination and tremor. Systemic injury with multi-organ involvement is reflected by systemic side effects, such as body weight loss, diarrhea, urination, and mortality (Barnes et al., 1996, Mohammad et al., 2008, Mohan et al., 2006).

Table 2.1: Shows the median lethal dose (LD_{50}) of cisplatin (Leite et al., 2012a)

Routes of administration	Animals	LD_{50}
Oral	Rat	25.5 mg/kg
Oral	Mouse	32.7 mg/kg
IV	Rat	21 mg/kg
IV	Mouse	20 mg/kg
IP	Rat	18.5 g/kg
IP	Mouse	17.4 mg/kg

2. Memantine: Acute Toxicity

From studies undertaken on the acute toxicity of memantine, in rats and mouse, they reported the following toxicity data (LD_{50}) (table 2.2). Clinical signs of intoxication included Ataxia, prone position and tremor preceded death (Barnes et al., 1996, Gad et al., 1988).

Table 2.2: Shows the median lethal dose (LD_{50}) of memantine (Stojiljković et al., 2019)

Routes of Administration	Animals	LD_{50}
Oral	rat	20 mg/kg
Oral	Mouse	18 mg/kg
IP	Rat	19 mg/kg
IP	Mouse	14 mg/kg
SC	rat	19 mg/kg
SC	mouse	17 mg/kg
IV	rat	18 mg/kg
IV	mouse	15 mg/kg

Chapter Three

MATERIALS AND METHODS

3.1. Instruments and Equipments

1. DANA 3200 ELISA Reader (Medical engineering Co. USA).

2. LISA 200 autoanalyzer (manufactured in France).

3. ABX Pentra 60 hematoanalyzer from HORIBA, USA.

4. Light microscopy (Leica, Germany).

5. Rotary microtome (Sakura. Acuu-Cut SRM200-Japan).

6. Spectrophotometer (Apeal PD-303-Japan).

7. Centrifuge (Centurion Scientific LTD., England).

8. Refrigerator (Bosch, Germany).

9. Shaker (Auto vortex SA6, UK.).

10. Electric sensitive balance (Mettler Toledo, Switzerland).

11. Paraffin embedding apparatus (Leica, Germany).

12. Manual camera (Toupcam ™, Japan).

13. Omni tissue homogenizer (10 mm) (Omni International, Inc, USA).

14. Water bath (Knauer, Germany).

15. Charged glass slides (Thermo Scientific™, USA).

16. Timer (stop-watch).

17. Pap pen (Dako, Germany).

18. Oven (Memmert, Germany).

19. Plastic test tube, scissors, scalpel, blade, cotton, insulin syringes, micropipette, tips of micropipette, coverslips, wash bottles, cylinders and flasks, gavage stainless steel needle for orally drenching of memantine drug, filter paper, ordinary glass slides, , surgical gloves, glass-staining jars, coplin jars and absorbent wipes.

3.2. Diagnostic Kits

1. Alkaline Phosphatase -Kit (ALP) (Vitro Scient Co., Hannover, Germany).

2. Blood Urea Nitrogen- Kit (BUN) (Vitro Scient Co., Hannover, Germany).

3. Serum Creatinine –Kit (Scr) (Vitro Scient Co., Hannover, Germany).

4. Creatine Kinase-Kit (CK) (Vitro Scient Co., Hannover, Germany).

5. Calcium, potassium, and sodium Diagnostic –Kit (Elabsciences Biotechnology Co., Ltd. USA).

6. Aspartate aminotransferase (AST) (Vitro Scient Co., Hannover, Germany).

7. Alanine aminotransferase (ALT) (Vitro Scient Co., Hannover, Germany).

8. Superoxide dismutase assay kit (SOD), Glutathione assay kit (GSH), Malondialdehyde assay kit (MDA), Glutathione peroxidase assay kit (GPx), Catalase assay kit (CAT) and Glutathione transferase assay kit (GST)(Elabsciences Biotechnology Co., Ltd. USA).

9. Dopamine ELISA kit of mice (D1R) (Elabsciences Biotechnology Co., Ltd. USA).

10. Serotonin ELISA kit of mice (ST/5-HT) (Elabsciences Biotechnology Co., Ltd. USA).

11. Acetyl cholinesterase ELISA kit of mice (AchE), Elabsciences Biotechnology Co., Ltd. USA.

12. Nicotinic acetylcholine receptors polyclonal antibody of anti-mouse (α7 nAChRs) (IHC). (E-AB-12583) (Elabsciences Biotechnology Co., Ltd. USA).

13. Myeloperoxidase Polyclonal Antibody of anti-mouse (MPO) (IHC). (E-AB-10466) (Elabsciences Biotechnology Co., Ltd. USA).

3.3. Animal Model

For this study adult male albino *Mus musculus* species, BALB/c strain mice which weighing 25-30 g of the same age (8-10 weeks) were used. They furnished with water and fed with standard diet pellet. Part of the study investigated at the laboratory house of the college of veterinary medicine, the other part which included fixation, processing, paraffin block, sectioning, staining by conventional hematoxylin and eosin stain (H&E stain), immunohistochemistry technique and serum analysis did at the chemical and histopathology laboratory of Shorsh hospital in Sulaimani governance.

Animals were maintained with conventional methods and were kept in plastic enclosures, on softwood fine granules as bedding. These were housed at 25 C with 12:12 h light: dark cycle. Experiments were started after two week of acclimatization period.

3.4. Drugs

1. Cisplatin sulphate100 mg/100 ml (Bristol-Myers Squibb, New York, NY, USA).

2. Memantine hydrochloride tablet 20 mg (Panpharma S. A., France). Memantine was dissolved in distilled water at 10mg/ml.

3. Xylazine 2% (Ceva Sante Animale -La Ballastere, France).

4. Ketamine hydrochloride 10% (Panpharma S. A., France).

3.5. Experimental Design

3.5.1. First Experiment: Acute Toxicity of cisplatin and memantine in male mice

In this study, one mouse was utilized for each dose. Different lethal doses of both cisplatin by intraperitoneal route and memantine by oral route were used.

Fifteen male mice were utilized, they were isolated according to a drug-treated group, result were recorded for each treatment after 24 hours and LD_{50} were calculated according to the up and down strategy (Bruce, 1985, Dixon et al., 2008).

1. Up and Down Method

This test calls for dosing singular creatures in arrangement separately at 24 hours intervals, with the underlying dose set at (the toxicologist best scale of LD_{50}) following every death state, the dose was decreased, while after every survival state, the dose was increased by predetermined dose change factor. If death follows an initial direction of upgrading doses or a survival follows an initial direction of diminishing

dose with the same ratio of the study. The LD_{50} is calculated using the following equation (Bruce, 1985, Dixon et al., 2008)

LD_{50} =xf +Kd

xf= last dose administered

K= value from Dixon table in the appendix [(1)]

d= difference between dose levels.

This experiment contains two groups divided according to the type of treatment:

1. Cisplatin sulphate: given by intraperitoneal injection, one mouse used for each dose in sequence.

2. Memantine hydrochloride: given by oral route, one mouse used for each dose in sequence.

3.5.2. Second experiment

The antagonistic effect of memantine hydrochloride to minimize the toxic side effects of cisplatin (nephrotoxicity, hepatotoxicity and hematotoxicity

A total of seventy-five adult male mice were allocated in plastic cages, in five groups of fifteen mice in each group, each weighing 25-30g. Mice were allocated into groups as follows:

1. Group 1 (G1): mice were treated orally with 1ml/kg distilled water for thirty days.

2. Group 2 (G2): mice were treated intraperitoneally with 4 mg/kg of cisplatin for thirty days (twice weekly).

3. Group 3 (G3): mice were treated orally with 5 mg/kg of memantine hydrochloride for thirty days (twice weekly).

4. Group 4 (G4): mice were pre-treated with 5 mg/kg of memantine hydrochloride and 4 mg/kg of cisplatin for thirty days (twice weekly). (cisplatin injection was carried out 1 hour post memantine administration).

5. Group 5 (G5): mice were pre-treated with 10 mg/kg of memantine hydrochloride and 4 mg/kg of cisplatin for thirty days (twice weekly). (cisplatin injection was carried out 1 hour post memantine administration).

At the end of the treatment period many biochemical analyses, histopathological staining of different organs in the body and immunohistochemical staining of α7 nAChRs and Myeloperoxidase MPO were done.

3.5.3. Third experiment

The neurobehavioral effect of both cisplatin and memantine in male mice

Fifty adult male were used in this experiment; ten mice for each experimental group. The neurobehavioral batteries of tests were considered weekly. Mice were allocated into groups as follows:

1. Group 1 (G1): mice were treated orally with 1ml/kg distilled water for thirty days

2. Group 2 (G2): mice were injected intraperitoneally with 4 mg/kg of cisplatin for thirty days (twice weekly).

3. Group 3 (G3): mice were treated orally with 5 mg/kg of memantine hydrochloride for thirty days (twice weekly).

4. Group 4 (G4): mice were pretreated with 5 mg/kg memantine hydrochloride and cisplatin 4 mg/kg for thirty days (twice weekly).(cisplatin injection was carried out 1 hour post memantine administration).

5. Group 5 (G5): mice were pretreated with 10mg/kg memantine hydrochloride and cisplatin 4 mg/kg for thirty days (twice weekly).(cisplatin injection was carried out 1 hour post memantine administration).

The following tests were done weekly during the treatment period:-

1. Open field activity

This test took place in a square wooden box (35x35x25 cm), the floor of which was divided into 25 squares equal in diameter. Rodents have an inborn curiosity to explore large open areas; the test therefore, provided simultaneous measures of locomotion, exploration, and anxiety by measuring the number of squares crossed by each mouse and recording the frequency of rearing, defecation, and urination in a period of 3 minutes (Jangra et al., 2016, Scholey and Kennedy, 2004, Macleod and van der Worp, 2010) (Figure 3.1)

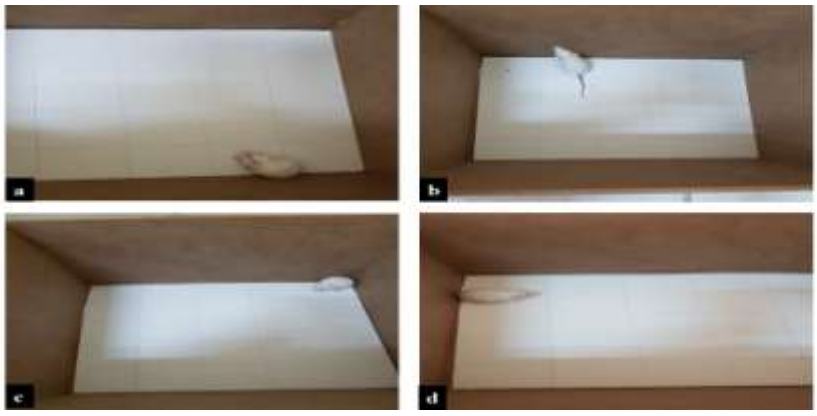

Figure 3.1: The open field box; (a,b,c,d) shows the different rearing state and position of the mice on drawing squares.

2. Negative geotaxis

This test measured vestibular function, neuromotor performance, and coordination. It involves a wooden stage sloped at 45 degrees: each mouse was placed in a head down position on the inclined surface and the time required for it to complete a 180-degree turn to correct its position in 60 seconds was then recorded (Jangra et al., 2016, Macleod and van der Worp, 2010) (Figure 3.2).

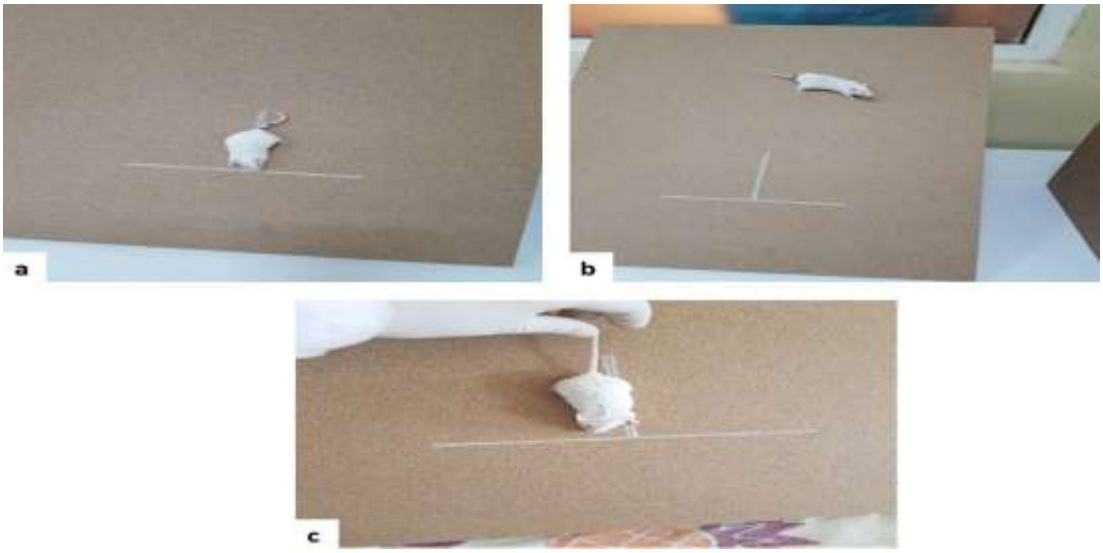

Figure 3.2: The negative geotaxis test; (a,b,c) shows the neuromotor performance, and coordination state on the slope

3. Hole-board test

The test was conducted on a circular wooden arena of diameter 30 cm and 10 cm height with 9 holes in the floor. The rodents explored these holes by pocking their heads (head dipping) (Mohammad et al., 2006, Mohan et al., 2006, Mohammad et al., 2007a, Prut and Belzung, 2003). The number of head pocks exhibited within a 3-minute period is inversely proportional to the degree of anxiety each animal feels (Figure 3.3).

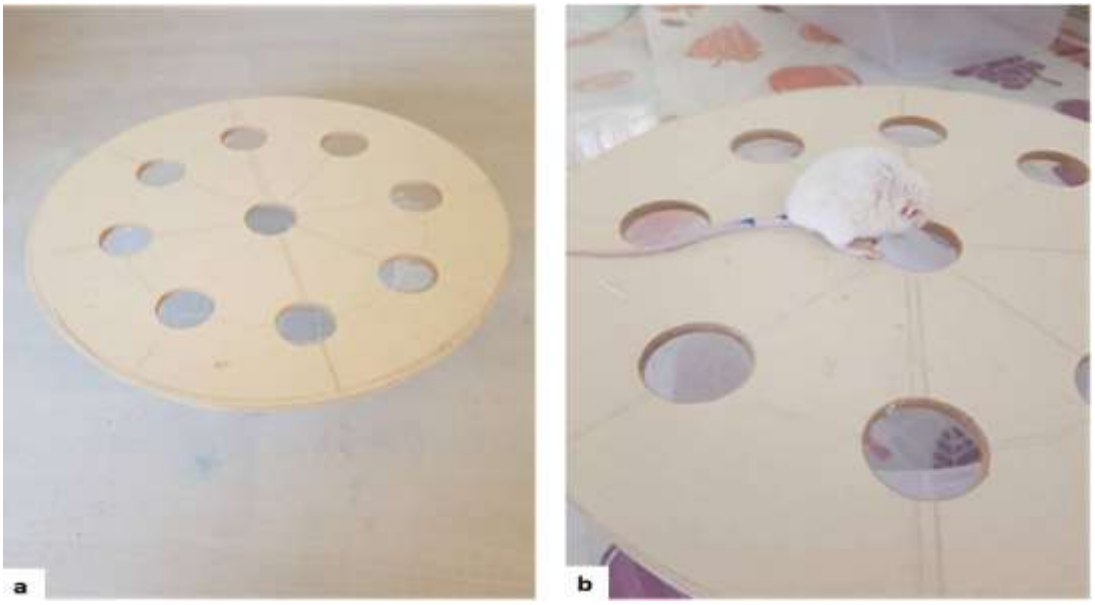

Figure 3.3:- Shows the hole-board test; both (a and b) shows the manually made board and the head dipping habits in the holes.

4. Swimming test

The animals were forced to swim in a cylinder height 30 cm and diameter 20 cm that contained water up to 18 cm deep (Figure 3.5). The cylinder was maintained at a temperature of 27 c for swimming duration of 10-15 seconds (Chtourou et al., 2015, Mohammad et al., 2006) and the swimming ability of each mouse was then graded as follows:-

Grade 1: - Nose under the water

Grade 2: - Nose and top of the head at or above water level and the ear still under water

Grade 3: - Nose is at or above water level and the water level reaches the center of the ear.

Grade 4:- Ear is fully above water level and the mice swim actively (Figure 3.4) (Mohammad et al., 2006, Dziewczapolski et al., 2009)

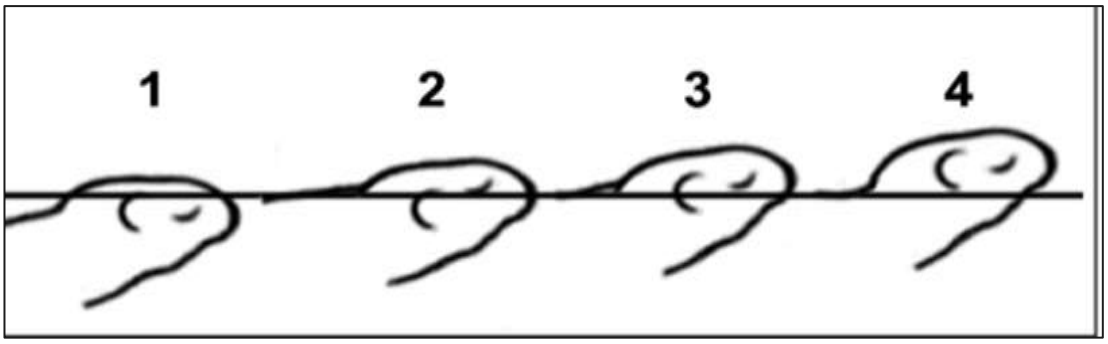

Figure 3.4: Shows the score of swimming ability of mice in the water with their grade starting from low grade to the high grade (Simonetti et al., 2009).

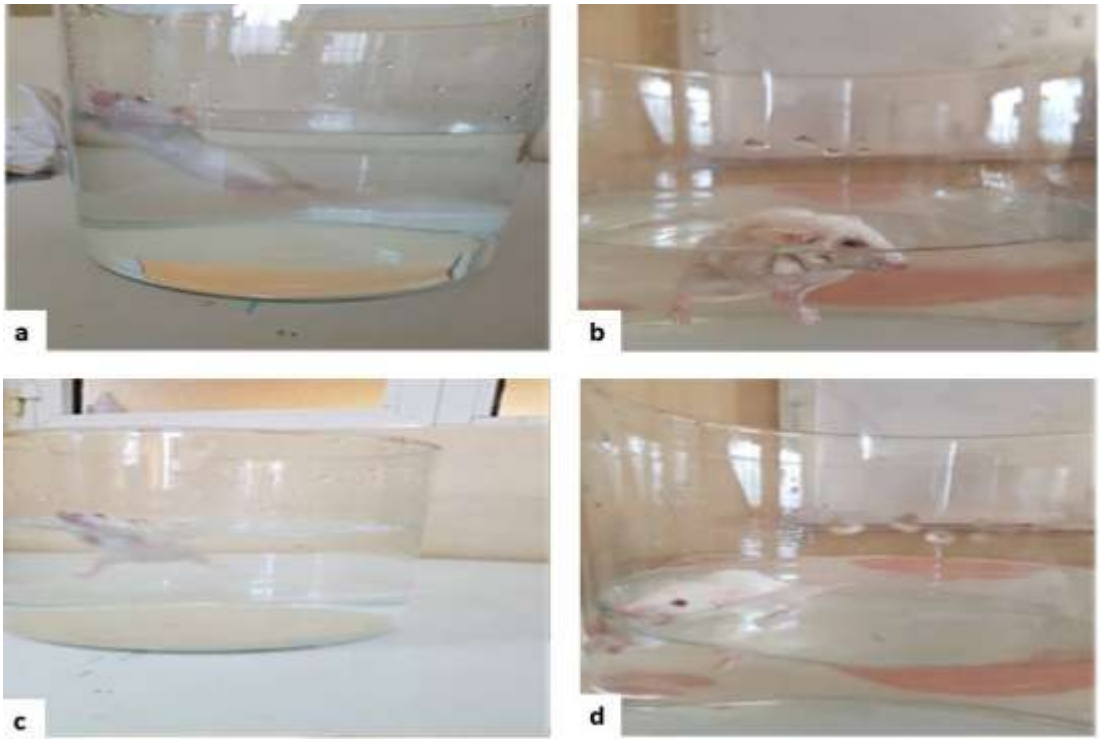

Figure 3.5: Shows the swimming test performance by each mouse from different treated group according to the main score of swimming ability.

3.6. Tissue sample preparation

Mice at the end of the experimental period, were euthanized by an intraperitoneal injection of xylazine and ketamine mixture (100 mg/kg Ketamine, 16 mg/kg Xylazine) followed by cervical dislocation (Schoell et al., 2009).

Organs from mice in each group including liver, brain, kidney, skeletal muscle, lung, heart, and testis were fixed in 10% buffered neutral formalin solution (100 ml formalin (40%), 4g of sodium phosphate monobasic, 6.5g of sodium phosphate dibasic and 900 ml D.W) and processed for paraffin embedding (Luna, 1968, Lipton, 2007). Two different sections of 4μm thickness were taken from each paraffin block of the liver, brain, kidneys, lung, muscle, heart and testis; the first section was mounted on an ordinary normal slide for hematoxylin and eosin stain and examined by different magnifying powers of light microscopy (Luna, 1968, Malik et al., 2006). The second section was mounted on a positively charged slide and subjected to the immunohistochemistry staining technique following the manufacturer's instructions supplied with the α7 nAChRs polyclonal antibody kit and myeloperoxidase Polyclonal Antibody (MPO) (Dziewczapolski et al., 2009, Abdelmeguid et al., 2010, Aracava et al., 2005).

3.7. Routine histopathological technique

Tissues such as heart, kidneys, liver, lung, skeletal muscle, brain and testis were processed as follows:

1- Fixation: Immediately after removal of the organs, each of them was taken and fixed in 10% neutral buffered formalin solution (10ml of 40% formaldehyde + 90ml of tap water), for 18-22 hours at room temperature.

2- Dehydration:- The tissues were expelled from the formalin (10%) and after that washed in running faucet water for 30 minutes to evacuate hints of fixative.

3- Clearing:- The aim of this step was to remove alcohol from tissue (dealcoholization).

4- Infiltration and embedding: - After clearing the tissues, they were passed through a mixture of xylene and molten paraffin wax (melting 56-58C°) for 30 minutes.

5- Tissue sectioning: - Rotary microtome with expendable cutting edges was utilized.

6- Tissue attachment: - Since formalin (10%) used as a fixative, therefore section adhesive was needed using Mayer's glycerol-albumin mixture.

7- De-wax and hydration: - De-wax was made by utilizing two changes of xylene (15 minutes for each change).

8- Staining: - Hematoxylin and Eosin staining were used.

9- Mounting: - This was made by utilizing DPX, coverslips were used to cover the sections (Luna, 1968).

3.8. Immunostaining method

3.8.1. Immunostaining procedure of anti-mouse polyclonal α7 nAChRs and anti-mouse polyclonal antibody Myeloperoxidase MPO.

1. Formalin-fixed paraffin embedded sections were cut into 4μm thickness for obtaining an optimum resolution and then placed on a positively charged slide to be stained.

2. The sections were baked in the oven (15 minutes at 56°C), then dewaxed in xylene (for 5-10 minutes).

3. The sections were rehydrated using graded alcohol (ethanol) in descending concentrations to water.

a. Absolute ethanol for 10 minutes.

b. 90% ethanol for 5 minutes.

c. 70 % ethanol for 5 minutes.

4. The sections washed under running tap water for 5 minutes, then placed in 3 changes of wash buffer (PBS), 2 minutes for each.

5. The slides were tapped off and the area around the specimen wiped to remove any remaining liquid and section encircled (wheeling) with pap pen.

6. Antigen retrieval was performed by adding a Few drops of previously prepared digestive enzyme pretreatment solution (1 vial lyophilized pepsin + 1 vial of reconstitution buffer were applied) to cover the specimen and incubated in a humid chamber for about 5 minutes at room temperature, then rinsed with wash buffer 3 times, 2 minutes for each.

7. Few drops of % hydrogen peroxide (endogenous peroxidase) blocking agent were applied to cover the specimen and incubated in a humid chamber for about 10 minutes at room temperature, then rinsed with wash buffer 3 times, 2 minutes for each

8. Few drops of protein blocking agent (bovine serum albumin) were applied to cover the specimen and incubated in a humid chamber for about 10 minutes at room temperature. Blot the slides without washing.

9. Incubation with 45 µl of α7 nAChRs polyclonal primary antibodies (rabbit anti-mouse) and MPO polyclonal primary antibodies (rabbit anti-mouse) (separately in each strategy for both antibody) in a humid chamber at room temperature for 1 hour followed by running in washing buffer (3×2 minutes).

10. Incubation with biotylinated secondary antibodies (goat anti-rabbit) in a humid chamber at room temperature for 30 minutes followed by running in washing buffer (3×2 minutes).

11. Incubation with horseradish peroxidase-streptavidin in a humid chamber at room temperature for 30 minutes followed by running in washing buffer (3×2 minutes).

12. Incubation with DAB + substrate - chromogen solution for 5-10 minutes in a humid chamber at room temperature followed by washing in running tap water for 5 minutes.

13. The slides were immersed in a bath of aqueous Hematoxylin for less than 1 minute and then rinsed gently under running tap water.

14. The slides were dehydrated consecutively by dipping in a glass jar containing the following:

a. 70% ethanol for 5 minutes.

b. 90% ethanol for 5 minutes.

c. Absolute ethanol for 10 minutes.

d. Xylene for 5-10 minutes.

15. Slides were mounted using a mounting medium (DPX) and covered with coverslips and left to dry (Chan, 2000, Aracava et al., 2005, Chen et al., 2010).

3.8.2. Interpretation of immunostaining results

1. Nicotinic acetylcholine receptors (α7 nAChRs)

Immunostaining of nicotinic acetylcholine receptors (α7 nAChRs) for the liver, muscle, and brain sections was scored by two independent readers on a scale from 0-4 where 0 = no staining, 1 = focal weak staining, 2 = focal medium staining, 3 = diffuse medium staining, 4 = diffuse strong staining (Song et al., 2008a).

2. Myeloperoxidase (MPO)

Immunohistochemical staining of myeloperoxidase among the renal parenchyma was evaluated by two independent pathologists. The myeloperoxidase staining was considered positive in cases of brown cytoplasmic reactivity in mesangial cells in glomeruli, epithelial cells of proximal and distal convoluted tubules with Henle loops, the process of immunohistochemistry staining done by following the procedure that was provided with the packs of polyclonal Myeloperoxidase MPO antibody (IHC). The power of immunostaining was freely scored on a scale extending from (0 – 3); evaluated 0-3; estimated as (0) no staining, (1) Weak staining, (2) Moderate staining and (3) Strong staining (Abdelmeguid et al., 2010, Aracava et al., 2005).

3.9. Blood samples collection

Blood samples were gathered from mice by heart puncture route, once time at the end of treatment period. Blood samples were partitioned into two parts; one part was collected in heparinized tubes contained EDTA as anticoagulant for the measurement of hematological parameters including red blood cells count (RBCs),

hemoglobin concentration (Hb%), white blood cells count (WBCs) and packed cells volume (PCV) concentration, while the other part was collected in tubes free of anticoagulants and centrifuged at 3000 rpm for ten minutes then the serum was collected in clean test tubes (Kumar and Gupta, 2011, Barbosa et al., 2008, Amin and Hamza, 2006).

3.10. Preparation of Tissue Homogenate

Mice were euthanized at the end of the treatment period; their livers were immediately extracted, set in chilled phosphate buffer solution (pH 7.4) at 4°C, blotted with filter paper and weighed. One gram was taken to prepare 10% of tissue homogenate using the same buffer solution and utilizing the Omni tissue homogenizer (10 mm). The homogenate was then centrifuged at 5000 rpm for ten minutes at 4°C (Kumar et al., 2014, Luqman et al., 2012); the homogenate level of lipid peroxide as MDA, GSH, GST, SOD, CAT and GPX contents were estimated by utilizing standard kits for each test (Kumar et al., 2011c, Kumar et al., 2011a, Torrelo et al., 2015b).

3.11. Experimental Parameters

1. Calculation of Animal's Weight

Animals from all groups were weighed weekly during the experimental period using a digital sensitive balance (Naqshbandi et al., 2012, Dziewczapolski et al., 2009).

2. Determination of blood parameters

Hemoglobin (Hb) concentration, red blood cells count (RBCs), packed cell volume (PCV) and white blood cells count (WBCs) were estimated using an automatic hematoanalyzer (Moore and Crom, 2006). The three main general principles used in hematoanalyzer are: electrical impedance, flow cytometry, and fluorescent flow cytometry. The conventional strategy utilized in almost every hematoanalyzer for counting cells is electrical impedance which has the ability to count RBCs, WBCs, PCV and Hb% (Weinstein et al., 2000b, Moore and Crom, 2006).

3. Determination of Blood Urea Nitrogen (BUN)

The method used Urease–Glutamate dehydrogenase, which is an enzymatic ultraviolet test carried out by auto analyzer LISA 200**.** The principle chemical equation of this test was (Mishra et al., 1977, Yokoo et al., 2009, Walser, 1998, Levinson, 1978).

$$Urea + 2H_2O \rightarrow 2NH_4 + 2HCO_3$$

$$Oxyglutarate + NH_4 + NADH \rightarrow L\text{-}Glutamate + NAD + H_2O$$

4. Determination of Serum Alkaline Phosphatase (ALP)

Serum alkaline phosphatase activity was determined according to the method of Kind and King (Kind and King, 1954) using ready-made kit depending on the liberation of the phenyl group which is calculated at 420 nm, reagents were diethanolamine, magnesium chloride and p- Nitrophenylphosphate (Proksch et al., 1973, Walser, 1998, Kind and King, 1954).

P-Nitrophenylphosphate +H2O →ALP→ phosphate + P.Nitrophenol

5. Determination of Serum Creatinine (Scr)

The principle of this test is that creatinine forms a colored orange complex in an alkaline picrate solution that carried out by auto analyzer LISA 200 by using ready-made kit Creatinine + picric acid → Creatinine picrate complex

Reagents were sodium hydroxide and picrate acid (Ali and Al Moundhri, 2006, Wang et al., 2004).

6. Determination of Serum Creatine Kinase (Ck)

The strategy of the test depends on the assurance of this chemical product by the ultra-violet test by using ready-made kit which carried out by auto analyzer LISA 200.

Creatine phosphate + ADP ↔CK↔ Creatine + ATP

Glucose + ATP ↔ HK↔ Glucose-6-phosphate + ADP

Glucose-6-phosphate + NADP ↔G6P-DH↔ Gluconate-6-phosphate + NADPH + H

(Johnsson and Wennerberg, 1999, Ali and Al Moundhri, 2006, Ali et al., 2008a).

7. Determination of Serum Alanine Aminotransaminase (ALT)

The most widely adopted clinical method in the determining the serum convergence of ALT by spectrophotometric detection, the measurement of the absorbance change of NADH concentration at 340 nm UV light depends on the

pyruvate reaction with lactate dehydrogenase (LDH) (Levinson, 1978, Washington and Van Hoosier, 2012).

8. Determination of Serum Aspartate Aminotransferase (AST)

The dimensions of aspartate aminotransferase (AST) in serum can help individuals analyze body tissues particularly the heart and the liver. This method based on Spectroscopic detection of continuously produced AST. Oxaloacetate delivered by AST from aspartate was dense with acetyl CoA to frame citrate and CoA in a framework combined with citrate synthase. The CoASH (reduced coenzyme A) formed was measured by its reaction with DTNB (5,5-dithio-bis-2-nitrobenzoic acid)(Washington and Van Hoosier, 2012).

3.12. Determination of lipid peroxidation and oxidative stress parameters in tissue homogenate:-

1. Determination of Malondialdehyde (MDA)

The final product of lipid peroxidation was examined by the method of Buege and Aust (Buege and Aust, 1978); which depend on the reaction of MDA with thiobarbituric acid to form MDA-thiobarbituric acid complex, a red chromophore which can be quantitated spectrophotometrically (Nair et al., 2007, Gawel et al., 2004).

2. Determination of Reduced Glutathione (GSH)

Total thiol bunches substance, which can be utilized as an indicator for reduced glutathione (GSH), was determined according to the strategy of Ellman (Ellman, 1959). The level of reduced glutathione (GSH) was determined on the basis of GSH oxidation with 5,5-dithiobis--nitrobenzoic acid (Scandalios, 2005, Eyer and Podhradský, 1986).

3. Determination of Glutathione peroxidase (GPx)

Glutathione Peroxidase is an enzyme which catalyzes the decrease of various peroxides; it protects the organisms from oxidative stress. The level of GPX is highly associated with the prevention, diagnosis and treatment of many diseases (Casanas-Sanchez et al., 2015, Jangra et al., 2016). Glutathione peroxidase (GPx) was estimated by the dithio-binitrobenzoic acid corrosive strategy, based on the response reaction between remaining glutathione after the action of GPx and 5,5-dithio bis-2-nitro benzoic acid) to form a complex which can be quantitated spectrophotometrically at 412 nm (Vareed et al., 2007, Nair et al., 2007).

4. Determination of Superoxide dismutase (SOD)

Superoxide dismutase was evaluated by the strategy given by Kakkar (Kakkar et al., 1984), which depend on the inhibition of formation of NADH-Phenazine methosulphate - nitro blue tetrazoliurn formazan. The intensity of the color that was formed can be quantitated spectrophotometrically at 560 nm.

5. Determination of Catalase (CAT)

Catalase was estimated by utilizing the strategy for Sinha (Dhanapal et al., 2010, Sinha, 1972). It depends on the principle that dichromate in acetic acid diminished to perchloric acid and chromic acetate in the presence of H202, as an unsteady intermediate. The absorbance of the chromic acetate produced was measured at 570 nm (Vareed et al., 2007).

6. Determination of Glutathione Transferase (GST)

Glutathione transferase activity test depended on the reaction of 2, 4 dichloronitrobenzene as a substrate which was assayed spectrophotometrically essentially as described by Hebig; the absorbance was read at 340 nm. GSTs activity was assayed at 25°C with reduced glutathione (GSH) and 1-chloro-2, 4-dinitrobenzene (CDNB) as substrates. The cuvettes in this test finally contained phosphate buffer (pH 6.5), GSH and chlorodinitrobenzene CDNB (Moatamedi Pour et al., 2014, Jin et al., 2007).

3.13. Determination of Acetylcholine Esterase (AChE)

Estimation of Acetyl cholinesterase (AChE) done by utilizing Sandwich-ELISA guideline, the microscale ELISA plate gave in this pack has been pre-covered with a counteracting agent explicit to Mouse AChE. samples are added to the miniaturized scale ELISA plate wells and joined with the particular immune response (Zhu et al., 2015). At that point, a biotinylated discovery counteracting agent explicit for Mouse AChE and Avidin-Horseradish Peroxidase (HRP) conjugate is added progressively to

each miniaturized scale plate well and hatched. Free parts are washed away. The substrate arrangement is added to each well. Just those wells that contain Mouse AChE, biotinylated location immune response and Avidin-HRP conjugate will seem blue in shading (Gohner et al., 2015). By adding the stop solution, the enzyme-substrate reaction be terminated, the optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm (Coull et al., 2005, Bocquené et al., 1990, Frasco et al., 2005) (Figure 3.8).

3.14. Determination of Dopamine (D1)

Measurement of dopamine is done by Sandwich-ELISA. Small scale ELISA plate furnished with the kit has been pre-covered with an antibody explicit to Mouse D1R. Standards or samples are added to the appropriate small ELISA plate wells and joined with the specific antibody. At that point, a biotinylated detection antibody explicit for Mouse D1R and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each microwell and incubated. Free parts are washed away. The substrate solution is added to each well. Just those wells that contain Mouse D1R, biotinylated detection antibody and Avidin-HRP conjugate will seem blue in color. The enzyme chemical -substrate reaction is ended by including stop solution. The optical density (OD) is measured spectrophotometrically at 450 nm (Nichkova et al., 2013, Carvour et al., 2008, Alaniz et al., 1999) (Figure 3.8)

3.15. Determination of Serotonin (ST/5HT)

Measurement of serotonin is done by Sandwich-ELISA. The microscale ELISA plate provided with the kit has been pre-covered with an antibody explicit to Mouse serotonin (Dong et al., 2007). Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for mice serotonin and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each microwell and incubated.

Standards or tests are added to the suitable small scale ELISA plate wells and joined with the particular counteracting antibody agent. At that point, a biotinylated identification antibody explicit for mice serotonin and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each smaller scale plate well and incubated. Free parts are washed away. The substrate solution is added to each well (Stockmeier et al., 1998, Kaye et al., 1998). Just those wells that contain mouse serotonin, biotinylated discovery immunizer and Avidin-HRP conjugate will seem blue in color, by including a Stop Solution, the chemical substrate response is ended and the color turns yellow. The optical density (OD) is estimated spectrophotometrically at 450 nm (Alaniz et al., 1999) (Figure 3.8)

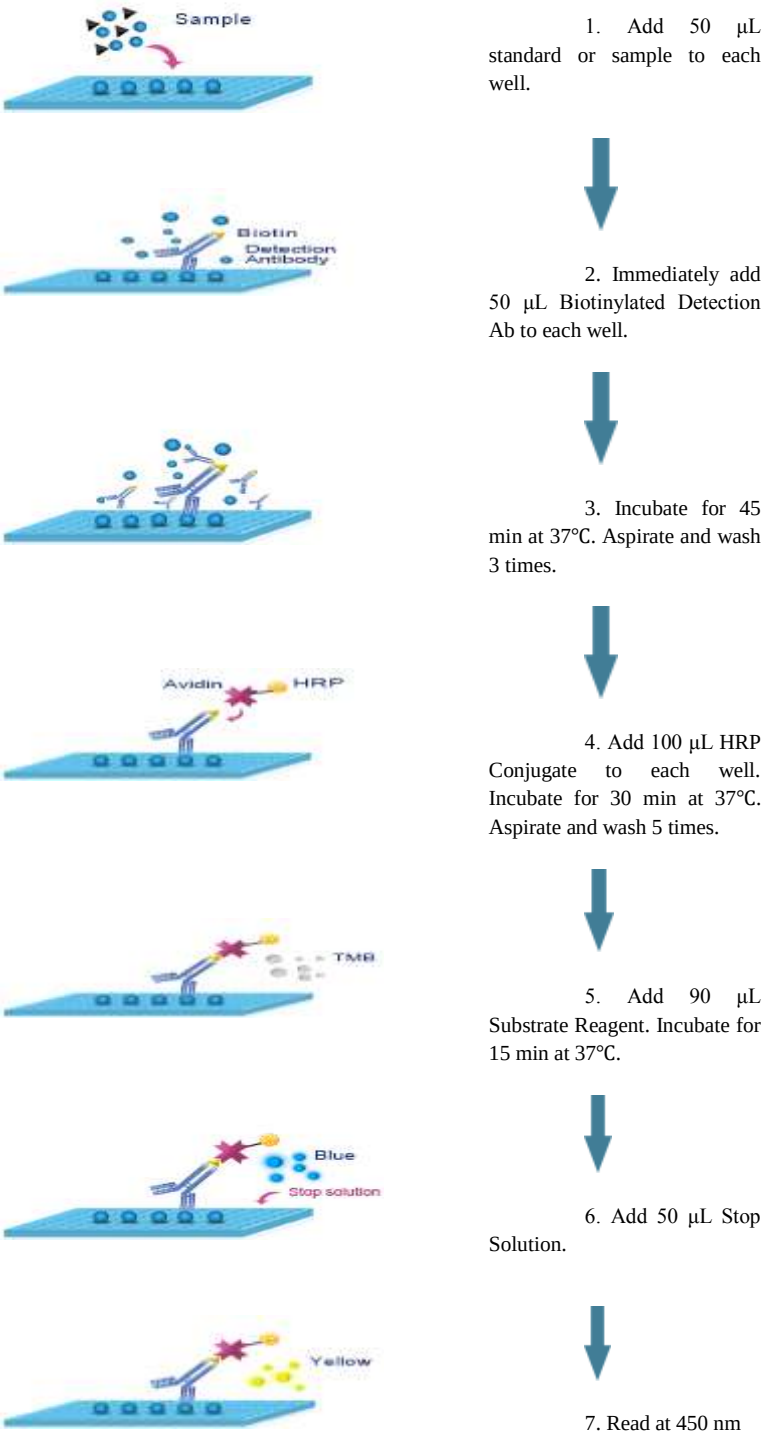

Figure (3.8): Shows the Procedure of ELISA technique for measuring Acetylcholine Esterase, Dopamine and Serotonin.

3.16. Electrolyte measurement

1. Calcium determination

Calcium Assay Kit gives a basic measure to decide calcium fixation inside the physiological samples like serum, plasma and tissue homogenate. Calcium ion in the sample binds to Methyl Thymol Blue (MTB) in alkaline solution and form a blue complex. Calcium content can be calculated by measuring the OD value at 610 nm (Guil et al., 1996, Willis, 1960).

2. Sodium determination

Sodium Assay Kit; Gives helpful two-step advance technique to identify sodium particles (Na+) present in serum. In this assay, sodium ions present in the sample are used by the enzyme β-galactosidase to produce o-nitrophenol an intermediate product, which generates a yellow color signal that can be detected at OD = 405 nm (Guil et al., 1996, Cohn and Dombrowski, 1971).

3. Potassium determination

Potassium Assay Kit provides a simple procedure for calculation of potassium level, potassium dependent pyruvate kinase catalyzes the change of NADH analog to NAD analog which is measured at 380 nm and is proportional to the potassium concentration (Guil et al., 1996, MacKinnon, 1991, Cohn and Dombrowski, 1971)

3.17. Statistical Analysis

Analysis was performed by utilizing One-Way Analysis Of Variance (ANOVA) and Duncan test to survey critical contrasts among treatment groups for the second test. While the third investigation done by Two-way ANOVA and Duncan for body weight and neurobehavioral tests. The measurable statistical level set at ($p<0.05$). Every examination was performed utilizing SPSS measurable form 21 programming variant (SPSS® Inc., USA).

CHAPTER FOUR

RESULTS

4.1. First Experiment: Measurement of LD_{50} of cisplatin and memantine

This experiment consists of 2 groups divided according to the type of treatment:

1. Cisplatin sulphate: acute toxicity result obtained from cisplatin treatment in mice by intraperitoneal route, by the up and down method as listed in (table 4.1). The range of doses was 10-16 mg /kg and the calculated value of LD_{50} was 15.48 mg/kg.

2. Memantine hydrochloride administered by oral route, the dose range was 10-18 mg/kg; the calculated value of LD_{50} was 17.48 mg/kg (table 4.2).

Table (4.1):- Shows the LD_{50} of cisplatin given in mice by up and down method

treatment	Animal used	Dose Range mg/kg	Difference between doses Mg/kg	Results after 24 hours	LD_{50} mg/kg
Cisplatin	7	10-16	2	oooxoxo	15.48

O=Survival X = Death

Table (4.2):- Shows the LD_{50} of memantine in mice by up and down method

Treatment	Animal used	Dose Range mg/kg	Difference between doses mg/kg	Results after 24 hours	LD_{50} mg/kg
Memantine	8	10-18	2	ooooxoxo	17.48

O=Survival X = Death

4.2. Second experiment

The antagonistic effect of memantine hydrochloride to minimize the toxic side effects of cisplatin (nephrotoxicity, hepatotoxicity and hematotoxicity)

4.2.1. Blood Urea Nitrogen Test (BUN)

Cisplatin treated group G2 caused a significant increase at the statistical level of ($P<0.05$) in the blood urea nitrogen at the end of treatment period (66.4±0.21), while in memantine treated group G3, the values of BUN showed insignificant change (31.1±0.31) in comparison to that of the (G1) control group (30.1±0.41) (table 4.3). The combination treatment G4 and G5 results of BUN levels are within the normal range (31.1±0.19) and (30.4±0.1) (table 4.3).

4.2.2. Serum Creatinine Test (Scr)

Cisplatin treated group G2 caused a significant increase at the statistical level of ($P<0.05$) in the serum creatinine level (3.4±0.03), however it slightly increased in G4 in combination treatment with low therapeutic dose of memantine 5mg/kg (1.9±0.1) in comparison with control group G1 (0.53±0.01) (Table 4.3) .The values of serum creatinine of those mice treated with combination treatment of high therapeutic dose of memantine 10mg/kg and cisplatin G5 showed insignificant changes in comparison to normal values in G1 (0.49±0.1). Mice treated with therapeutic dose of memantine (G 3) showed insignificant change in the level of serum creatinine (0.5±0.41) (table 4.3).

4.2.3. Alkaline Phosphatase Test (ALP)

Mice treated with cisplatin G2 reveals a significant increment in ALP at the statistical level of ($P<0.05$) (144.1±0.21) (table 4.3). The combination treatment of memantine 10 mg/ kg and cisplatin group G5 and memantine treated group alone in G3 showed insignificant changes in the ALP levels (66.1±0.1) and (65.5±0.14) relatively similar to those values obtained from control group G1 (65.4±0.43), significant increase in pretreatment group of memantine 5mg/kg and cisplatin G4 (75.31±0.71) (table 4.3).

4.2.4. Alanine aminotransferase (ALT)

Cisplatin treated group G2 showed a significant increase at the statistical level of ($P<0.05$) in the serum ALT level (63.19±0.78) (Table 4.3). Those mice pretreated with either low or high therapeutic dose of memantine 5 mg/kg and 10mg/kg respectively with a therapeutic dose of cisplatin 4 mg/kg, showed insignificant changes and the values are within normal range of control group (38. 9±0.91) and (38.1±0.81) respectively for G4 and G5. Mice treated with therapeutic dose of memantine G3 showed values within normal range (38. 8±0.64) (table 4.3)

4.2.5. Aspartate aminotransferase (AST)

Mice treated with both doses 5mg/kg and 10 mg/kg of memantine G4 and G5 showed insignificant effect on the AST at the statistical level of ($P<0.05$) (59.91±0.19) (58.71±0.1) similar to that of control group G1 (58.91±0.41) (table 4.3). Those mice treated with a therapeutic dose of cisplatin alone G2, showed a significant increase in AST level (126.41±0.21) in comparison with G1 (table 4.3).

4.2.6. Creatine kinase (Ck)

Cisplatin treated group G2 caused a significant increase at the statistical level of (*P<0.05*) in the serum CK (5.9 ± 0.3) and significantly increased in the memantine combination treated group with cisplatin in G4 (2.80 ± 0.9) in comparison with values in G1 (1.5 ± 0.4). The values of CK in G5 showed insignificant changes in comparison with G1 (1.6 ± 0.2) (table 4.3).

Table 4.3: Shows the effect of cisplatin and memantine on renal and liver parameters

Parameter / Groups	Ck (mg/dl)	Scr (mg/dl)	ALP (IU/L)	ALT (IU/L)	BUN (mg/dl)	AST (IU/L)
G1	1.5 ± 0.4 a	0.3±0.01 a	65.4±0.43 a	38.3±0.91 a	30.1±0.41 a	58.9±0.41 a
G2	5.9 ± 0.3 c	3.4±0.03 c	144.1±0.21 c	63.9±0.78 b	66.4±0.21 b	126.1±0.21 b
G3	1.4 ± 0.1 a	0.5±0.41 a	65.5±0.14 a	38. 8±0.64 a	31.1±0.31 a	58.9±0.31 a
G4	2.8 ± 0.9 b	1.9±0.1 b	75.1±0.71 b	39. 1±0.81 a	31.3±0.19 a	59.1±0.19 a
G5	1.6 ± 0.2 a	0.4±0.1 a	66.1±0.1 a	38. 9±0.91 a	30.4±0.1 a	59.7±0.1 a

n=15 mice, values in the table expressed as mean ± SE (standard error), different letters mean significant variation at *P< 0.05*

4.2.7. Serum Calcium, Sodium and Potassium

The use of therapeutic dose of cisplatin sulphate G2 had a significant decreasing effect on the serum calcium, sodium and potassium levels (5.14±0.19), (3.0±0.79) and (0.84±0.89) in comparison with that of the control group G1 (7.44±0.25), (4.4±0.25) and (2.19±0.71) respectively. The lower therapeutic dose of memantine G4 showed significant decrease in serum calcium and potassium (5.09±0.10) and (3.64±0.10), while the higher therapeutic dose of memantine showed insignificant changes in calcium, potassium and sodium levels in comparison with G1. Groups of mice treated with memantine alone showed insignificant changes in the serum levels of calcium, potassium and sodium after treatment period (table 4.4).

Table 4.4: Effect of cisplatin and memantine on serum level of calcium, potassium and sodium

parameters / Groups	Calcium	potassium	Sodium
G1 Control	7.0±0.5 a	4.4±0.2 a	2.19±0.7 a
G2 cisplatin	5.4±0.19 b	3.0±0.2 b	0.94±0.8 b
G3 Memantine 5mg/kg	6.8±0.8 a	4.0±0.3 a	2.01±0.2 a
G4 Cisplatin 4mg/kg and memantine 5mg/kg	5.9±0.19 b	3.9±0.5 a	1.99±0.5 a
G5 Cisplatin 4 mg/kg and memantine 10 mg/kg	6.40±0.37 a	4.1±0.67 a	2.14±0.11 a

n=15 mice, values in the table expressed as mean ± SE (standard error), different letters mean significant variation at $P<0.05$

4.2.8. Tissue homogenate content of enzymatic and non-enzymatic antioxidants parameters

The level of MDA is significantly high at ($P<0.05$) in cisplatin-treated group G2 (5.4±0.89) when compared with values of the control group G1(0.9±0.71), memantine treatment alone G3 (1.1±0.29) showed dropped level of MDA in comparison with G2 group (table 4.5). Cisplatin treated group G2 significantly increased lipid peroxidation and decreased GSH, SOD, GPX, CAT and GST level as enzymatic and non-enzymatic anti-oxidant parameters in regard to the values in G1, while in G3 and a combination treatments of high therpeutic dose of memantine G5 the levels of MDA, GPX, GST, SOD, CAT and GSH showed insignificant changes in regard to G1(Table 4.5). Pretreated mice with low therapeutic dose of mementine and cisplatin G4 showed significant decrease in the level of GSH (14.4±0.10), GPX (1.74±0.10) and catalase (6.31±0.71) in comparison with values in control group G1.

Table 4.5: Effect of cisplatin and memantine on enzymatic and non-enzymatic antioxidants parameters

Groups / parameters	G1 Control group	G2 Cisplatin 4mg/kg	G3 Memantine 5mg/kg	G4 Cisplatin 4mg/kg and memantine 5mg/kg	G5 Cisplatin 4mg/kg and memantine 10mg/kg
MDA nmol/g tissue	0.9±0.71 a	5.4±0.89 b	1.1±0.29 a	1.3±0.59 a	1.4±0.11 a
GSH µmol/g Tissue	18.4±0.25 a	11.4±0.79 c	17.9±0.10 a	14.4±0.10 b	18.9±0.67 a
GST (U/g tissue)	70.4±0.43 a	44.11±0.21 b	69.6±0.14 a	66.31±0.71 a	69.1±0.1 a
SOD nmol/g tissue	2.9±0.71 a	0.94±0.89 b	2.41±0.29 a	2.3±0.59 a	2.4±0.11 a
Gpx Glutathione peroxidase (U/g tissue)	2.19±0.71 a	0.64±0.89 c	2.01±0.29 a	1.74±0.10 b	1.98±0.37 a
Catalase (U/g tissue)	8.5±0.25 a	4.4±0.79 b	8.4±0.3 a	6.31±0.71 b	7.9±0.77 a

n=15 mice, values in the table expressed as mean ± SE (standard error), different letters mean significant variation at $P<0.05$

4.2.9. Hematological parameters

Cisplatin treated group G2 showed a significant increase in WBCs (10.41±0.21) and a decrease in RBCs (5.7±0.65), PCV (20.41±0.21) and Hb (3.91±0.21), while in combination treatment group of both low therapeutic and high therapeutic doses of memantine G4 and G5 showed values similar to those of control group G1, RBC (7.3±0.9), WBC (8.50±0.41), Hb (5.14±0.43) and PCV (28.50±0.41) (Table 4.6).

Table 4.6: Effect of cisplatin and memantine on blood parameters

parameters / Groups	RBC X 10^{6} ML	WBC X 10^{3} ml	HB g/dl	PCV %
G1 Control	7.5±0.9 a	8.50±0.41 a	5.14±0.43 a	28.50±0.41 a
G2 Cisplatin 4mg/kg	5.7±0.65 b	10.41±0.21 b	3.91±0.21 b	20.41±0.21 b
G3 Memantine 5mg/kg	7.6±0.526 a	8.61±0.317 a	5.21±0.145 a	28.31±0.312 a
G4 Cisplatin 4mg/kg and memantine 5mg/kg	7.6±0.936 a	8.58±0.197 a	5.31±0.71 a	28.41±0.19 a
G5 Cisplatin 4mg/kg and memantine 10mg/kg	7.6±0.1 a	8.61±0.18 a	5.40±0.1 a	28.71±0.128 a

n=15 mice, value in the table expressed as mean ± SE (standard error), different letters mean significant variation at $P<0.05$

4.2.10. Serum Acetyl cholinesterase Level

Cisplatin treated group G2 caused a significant increase at the statistical level of ($P<0.05$) of AchE level (34.82 ± 2.2) in comparison with those treated with memantine alone G3 (21.9 ± 0.11) and control group G1 (25.08 ± 3.2) (Table 4.7). Those mice treated with a combination treatment of either low and high therapeutic dose of memantine with cisplatin G4 and G5 showed an insignificant changes in comparison with values in G1 (23.42 ± 2.31), (25.60 ± 0.20).

4.2.11. Serum Serotonin Level

Cisplatin treated group G2 caused a significant decrease at the statistical level of ($P<0.05$) in the serum serotonin level (1.01±0.21) in comparison with those treated with memantine alone (1.44±0.31) and control group (1.41±0.41) (Table 4.7). Those mice treated with both a combination treatment of low and high therapeutic dose of memantine with cisplatin G4 and G5 showed an insignificant change (1.21±0.19) and (1.31±0.1).

4.2.12. Serum Dopamine Level

Cisplatin treated group G2 caused a significant increase at the statistical level of ($P<0.05$) in dopamine level (3.01±0.1) in comparison with those treated with memantine alone G3 (1.17±0.14) and control group G1 (1.24±0.53) (Table 4.7). Mice treated with a combination treatment of either low or high therapeutic dose of memantine with cisplatin G4 and G5 showed an insignificant changes (1.30±0.71) and (1.32±0.1) in comparison with values of G1.

Table 4.7: Effect of cisplatin and memantine on serum acetyl cholinesterase, serotonin and dopamine

parameters / Groups	AchE pmol/mL	Serotonin pmol/ mL	Dopamine pmol/ mL
G1 Control	25.08 ± 3.2 a	1.41±0.41 a	1.24±0.53 a
G2 Cisplatin 4 mg/kg	34.82 ± 2.2 b	0.91±0.21 b	3.01±0.1 b
G3 Memantine 5 mg/kg	24.9 ± 0.11 a	1.64±0.31 a	1.17±0.1 a
G4 Cisplatin 4 mg/kg and memantine 5 mg/kg	24.42 ± 2.31 a	1.51±0.19 a	1.30±0.21 a
G5 Cisplatin 4 mg/kg and memantine 10 mg/kg	24.60 ± 0.20 a	1.44±0.1 a	1.32±0.1 a

n=15 mice, values in the table expressed as mean ± SE (standard error), different letters mean significant variation at $P<0.05$

4.3. Third experiment: The neurobehavioral effect of both cisplatin and memantine alone and in combination

4.3.1. Open field activity

A. Number of squares crossed

Cisplatin treated mice G2 showed a significant decrease at the statistical level of (*P<0.05*) in the number of squares crossed in the open field box after the second week of treatment (28.41±0.31), this decline continued to the end of the treatment period in comparison with control group G1 (29.11± 0.31). The lower therapeutic dose of memantine with cisplatin treatment group G4 showed significant changes in the last week of the treatment period (25.91±0.16). The other groups; G3 (28.31±0.41) and G5 (29.71±0.1) showed insignificant changes (table 4.8).

Table 4.8: Shows the squares crossed in the open filed test

Weeks / Groups	1st week	2nd week	3rd week	4th week
G1 Control	29.11± 0.31 a	30.01±0.41 a	29.91±0.13 a	29.81±0.18 a
G2 Cisplatin 4mg/kg	28.41±0.31 a	24.21±0.21 b	23.31±0.51 b	15.11±0.16 c
G3 Memantine 5mg/kg	28.31±0.41 a	28.71±0.31 a	28.11±0.14 a	25.51±0.14 b
G4 Cisplatin 4mg/kg and memantine 5mg/kg	28.71±0.1 a	27.41±0.19 a	27.71±0.71 a	25.91±0.16 b
G5 cisplatin 4 mg/kg and memantine 10/kg	29.71±0.1 a	29.41±0.19 a	28.71±0.71 a	28.51±0.16 a

n=10 mice, values in the table expressed as mean ± SE (standard error), different letters mean significant variation at *P<0.05*

B. Frequency of rearing

Cisplatin treated group G2 showed significant decrease at the statistical level of (*P<0.05*) in frequency of rearing in the open field test after the second week of treatment (9.1± 0.5), this dropping continued to the end of treatment period and showed significant changes in comparison with values of G1(11.8 ± 0.3). The lower therapeutic dose of memantine with cisplatin treatment group G4 showed significant changes in the third and fourth week of the treatment period (9.19 ± 0.6) and (6.91±0.6) G3 (11.5 ±0.4) showed insignificant changes frequency of rearing and resemble the control group for four weeks while G5 (11.7 ±0.2) only in the last week showed significant changes in comparison with control group (table 4.9).

Table 4.9: Shows the frequency of rearing in the open field test

Weeks / Groups	1st week	2nd week	3rd week	4th week
G1 Control	11.8 ± 0.3 a	11.4 ± 0.81 a	11.3 ± 0.33 a	11.51±0.3 a
G2 Cisplatin 4mg/kg	11.3 ± 0.5 a	9.1± 0.5 b	6.1 ± 0.91 c	3.1 ± 0.2 d
G3 memantine 5mg/kg	11.5 ±0.4 a	11.1 ± 0.9 a	10.7 ± 0.84 a	9.1 ± 0.4 b
G4 cisplatin 4mg/kg and memantine 5mg/kg	10.9 ±0.1 a	10.8 ± 0.8 a	9.19 ± 0.6 b	6.91±0.6 c
G5 cisplatin 4mg/kg and memantine 10mg/kg	11.7 ±0.2 a	11.1 ± 0.6 a	10.23 ± 0.4 a	9.91±0.6 b

n =10 mice, values in the table expressed as mean ± SE (standard error), different letters mean significant variation at *P<0.05*

C. Frequency of urination and defecation

Cisplatin treatment group G2 showed significant increase at the statistical level of (*P<0.05*) in frequency of urination and defecation in the open field test after the second week of treatment (5.41±0.77), this state continued and showed significant changes in comparison with control group G1(4.1±0.31). The lower therapeutic dose of memantine with cisplatin treatment group G4 showed insignificant changes during the treatment period (4.0±0.1)(table 4.10) The other group like G3 (4.08±0.41) showed insignificant changes in urination and defecation frequency as those of control group G5 (4.60±0.1).

Table 4.10: Shows the urination and defecation frequency in the open field test

Weeks / Groups	1st week	2nd week	3rd week	4th week
G1 control	4.1±0.31 a	4.41±0.81 a	4.31±0.9 a	4.51±0.33 a
G2 Cisplatin 4mg/kg	4.41±0.31 a	5.41±0.77 b	5.71±0.77 b	5.91±0.77 b
G3 Memantine 5 mg/kg	4.08±0.41 a	4.11±0.77 a	4.41±0.14 a	4.09±0.94 a
G4 cisplatin 4mg/kg and memantine 5 mg/kg	4.0±0.1 a	4.9±0.89 a	4.55±0.61 a	3.91±0.16 a
G5 cisplatin 4 mg/kg and memantine 10 mg/kg	4.60±0.1 a	4.3±0.89 a	4.15±0.61 a	4.94±0.16 a

n=10 mice, values in the table expressed as mean ± SE (standard error), different letters mean significant variation at *P<0.05*

4.3.2. Hole-board test

Cisplatin treatment group G2 showed significant decrease at the statistical level of (*P<0.05*) in the number of head dipping after the second week of treatment (8.4±0.51), in comparison with the values of control group G1(11.3±0.31). The lower therapeutic dose of memantine with cisplatin treatment group G4 showed significant decrease in the second week of treatment period (9.7±0.49)(table 4.11).G3 memantine treated mice (9.91±0.44) showed significant changes in the third week of treatment, while those mice in G5 only showed slight decrease in the last week of treatment period (9.1±0.67).

Table 4.11: Shows the frequency of hole bored test

parameters / Groups	1st week	2nd week	3rd week	4th week
G1 control	11.3±0.31 a	11.5±0.81 a	11.6±0.9 a	11.4±0.33 a
G2 Cisplatin 4mg/kg	10.3±0.31 a	8.4±0.51 b	4.9±0.17 c	3.1±0.56 c
G3 memantine 5mg/kg	11.8±0.41 a	10.9±0.79 a	9.91±0.44 b	9.1±0.67 b
G4 Cisplatin 4mg/kg and memantine 5 mg/kg	10.9±0.26 a	9.7±0.49 b	9.45±0.71 b	8.81±0.36 b
G5 Cisplatin 4mg/kg and memantine 10 mg/kg	10.8±0.71 a	11.1±0.79 a	10.91±0.44 a	9.1±0.67 b

n = 10 mice, values in the table expressed as mean ± SE (standard error), different letters mean significant variation at *P<0.05*

4.3.3. Negative geotaxis

Cisplatin treated group G2 showed significant increase at the statistical level of (*P<0.05*) in the time required by mice for body correction and to do the negative geotaxis test after the second week of treatment (3.4±0.76), in comparison with control group G1(1.19±0.19). The lower therapeutic dose of memantine with cisplatin treatment group G4 showed significant changes in the third week of treatment period (3.1±0.74) (table 4.12). Memantine treated mice G3 (1.1±0.81) showed insignificant changes in doing negative geotaxis and similar that of control group G5 (1.2±0.06).

Table 4.12: Shows the results of negative geotaxis test

parameters / Groups	1st week	2nd week	3rd week	4th week
G1 control	1.19±0.19 a	1.2±0.81 a	1.1±0.9 a	1.4±0.33 a
G2 Cisplatin 4mg/kg	1.3±0.44 a	3.4±0.76 b	6.9±0.17 c	7.1±0.06 c
G3 Memantine 5mg/kg	1.1±0.81 a	1.4±0.79 a	1.5±0.44 a	1.6±0.87 a
G4 Cisplatin 4 mg/kg and memantine 5 mg/kg	1.3±0.06 a	1.9±0.39 a	3.1±0.74 b	4.1±0.36 b
G5 Cisplatin 4mg/kg and memantine 10 mg/kg	1.2±0.06 a	1.09±0.39 a	1.11±0.74 a	1.31±0.36 a

n = 10 mice, values in the table expressed as mean ± SE (standard error), different letters mean significant variation at *P<0.05*

4.3.4. Swimming

Cisplatin treated group G2 showed a significant decrease at the statistical level of (*P<0.05*) in the score of swimming ability after the second week of treatment (score 3), this decrease continued until it reached score (1) in the last week of treatment, and showed significant changes in comparison with control group G1 (score 4). The lower therapeutic dose of memantine with cisplatin treatment group G4 showed significant changes from the second week of the treatment (score 3)(table 4.13). The other group included G3 (score 4) showed insignificant changes in swimming score and resemble that of control group, while G5 showed slight significant decrease in the last week (score 3).

Table 4.13: Shows the results of the swimming test

Weeks / Groups	1st week	2nd week	3rd week	4th week
G1 Control	4± 0.2 a	4±0.2 a	4 ±0.9 a	4± 0.3 a
G2 Cisplatin	4± 0.1 a	3±0.1 b	2±0.5 c	1 ± 0.6 d
G3 memantine	4 ± 0.3 a	4 ±0.5 a	4 ±0.3 a	4 ±0.67 a
G4 Cisplatin 4mg/kg and memantine 5 mg/kg	4 ± 0.6 a	3 ±0.3 b	3 ±0.2 b	2 ±0.3 c
G5 cisplatin 4 mg/kg and memantine 10 mg/kg	4 ±0.2 a	4 ±0.6 a	4 ±0.11 a	3 ±0.1 b

n = 10 mice, values in the table expressed as mean ± SE (standard error), different letters mean significant variation at *P<0.05*

4.3.5. Calculation of animal's weight

Mice treated with cisplatin G2 showed significant decrease in the weight after the second week of treatment (25.4±0.76), this drooping in the weight continued to the end of treatment period and showed obvious changes in comparison with mice in G1(30.4±0.1). The lower therapeutic dose of memantine with cisplatin treatment group G4 showed significant changes in the fourth week of the treatment period (25.1±0.66). Mice in G3 (29.1±0.81) showed insignificant changes in weights of animals and compatible with those values of G1, G5 (30.10 ±0.8) showed insignificant changes in comparison with control group (table 4.14).

Table 4.14: Shows the results of the animal weights in gram

parameters / Groups	1st week	2nd week	3rd week	4th week
G1 Control	30.4±0.19 a	30.2±0.81 a	29.91±0.9 a	29.94±0.33 a
G2 Cisplatin 4 mg/kg	29.3±0.44 a	25.4±0.7 b	23.9±0.17 c	20.1±0.06 c
G3 Memantine 5 mg/kg	29.1±0.81 a	28.4±0.79 a	28.1±0.44 a	28.2±0.87 a
G4 Cisplatin 4mg/kg and memantine 5 mg/kg	30.3±0.06 a	29.9±0.37 a	28.4±0.18 a	25.1±0.66 b
G5 Cisplatin 4 mg/kg and memantine 10 mg/kg	30.10 ±0.8 a	29.9±0.19 a	29.4±0.38 a	28.7±0.26 a

n=10 mice, values in the table expressed as mean ± SE (standard error), different letters mean significant variation at $P<0.05$

4.4. Histopathological lesions by H&E stain:-

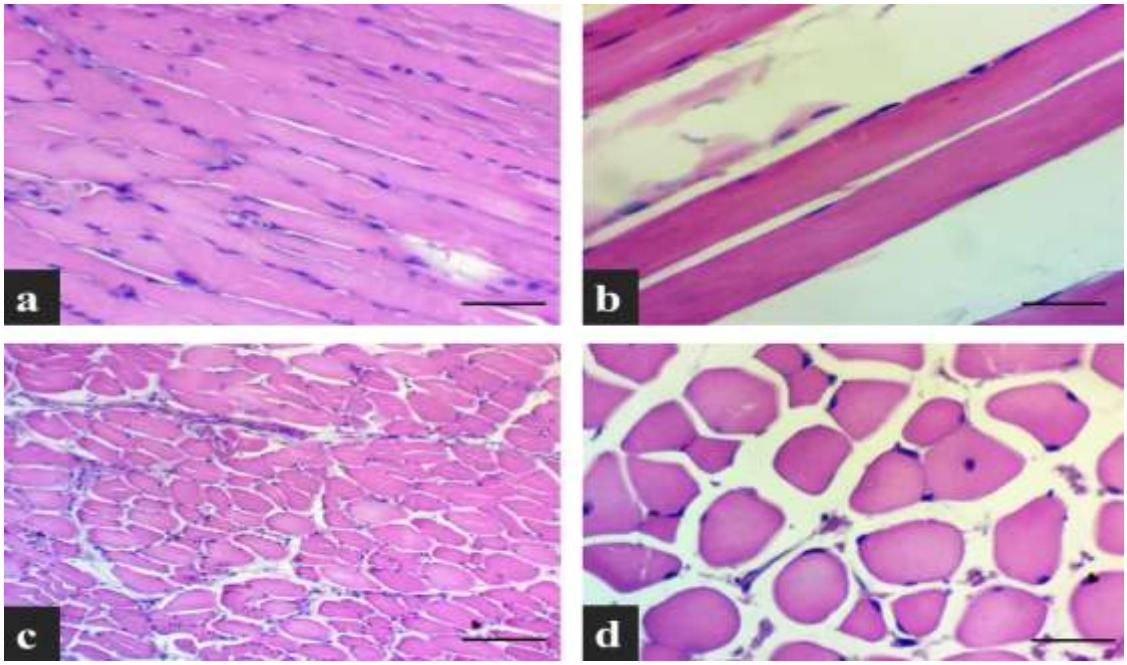

Figure 4.1:- Microscopical section of skeletal muscle in control group G1. a and b: Normal histological features of longitudinal section of skeletal muscle, multi-peripheral located nuclei with normal striation, (H&E stain, scale bar 100 µm, scale bar 20 µm), c and d: Transvers section of skeletal muscle showed normal histological structures, (H&E stain, scale bar 100 µm, scale bar 20 µm).

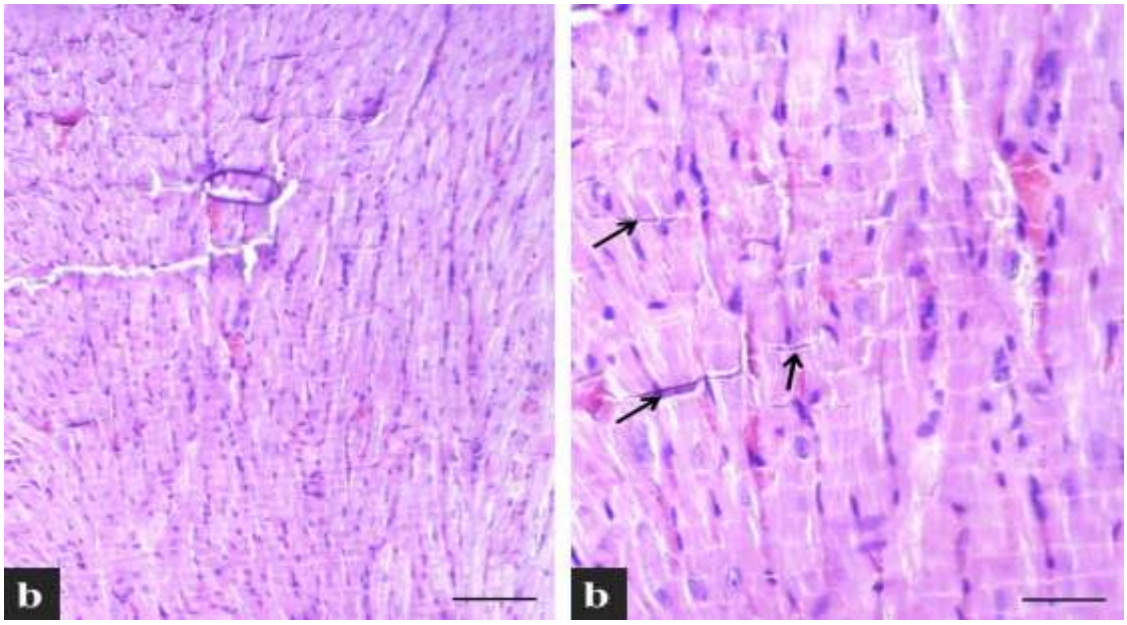

Figure 4.2:- Microscopical section of cardiac muscle in control group G1 a-b: Normal histological arrangement with intact sarcoplasm and nuclei, normal intercalated disc structures as indicated by black arrows, (H&E stain, scale bar 100 µm, scale bar 20 µm).

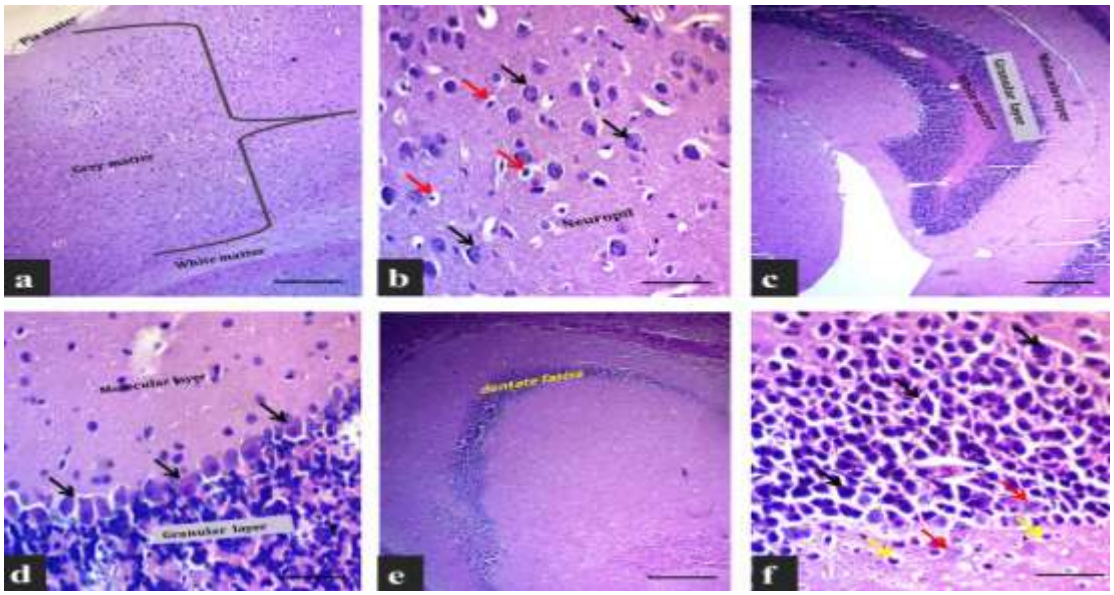

Figure 4.3:- Microscopical section of the brain sections of control group G1. a and b: The cerebrum showed normal layers architecture, neurons (black arrows), and glial cells as indicated by red arrows were showed intact morphology, (H&E stain, scale bar 100 µm, scale bar 20 µm), c and d: The cerebellar layers have normal histological appearance, and purkenji cells (black arrows) were in normal histological features (H&E stain, scale bar 50 µm, scale bar 20 µm), e and f: In the hippocampus section the neurons (black arrows), cortical cells (red arrows), and glial cells as indicated by yellow arrows showed closely arranged, prominent nuclei, with intact morphology, (H&E stain, scale bar 100 µm, scale bar 20 µm).

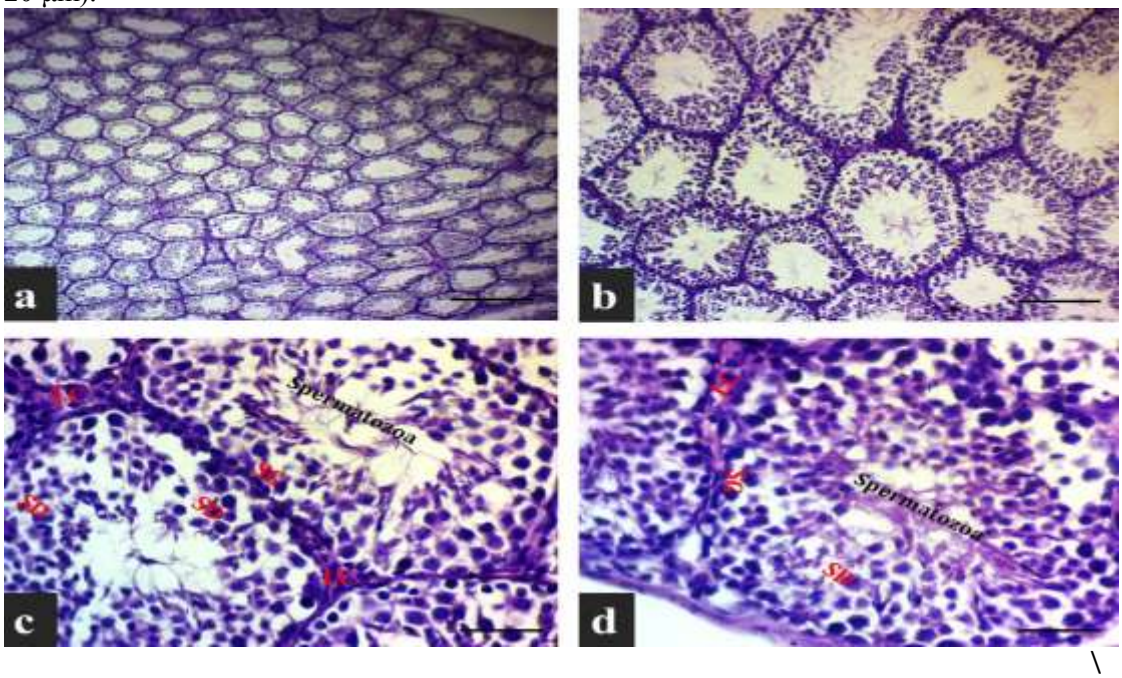

Figure 4.4: Microscopical section of seminiferous tubules (Testis) sections in control group G1. a and b: Normal arrangement of seminiferous tubules at different spermatogenic stages and spermatozoa without any damages, (H&E stain, scale bar 100 µm, scale bar 50 µm), c and d: Normal histological structures of spermatogonia (SG), spermatide (SD), spermatozoa, and interstitial cells (Lediyg cells) , (H&E stain, scale bar 20 µm),

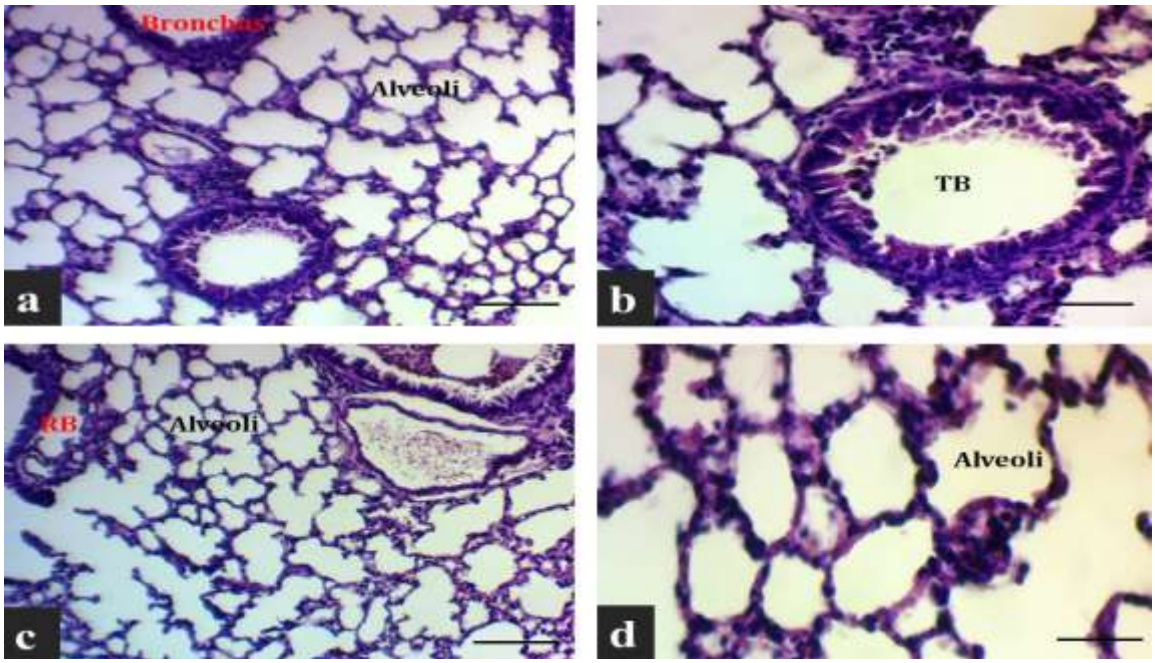

Figure 4.5: Microscopical section of lung section in control group G1. a and b: Normal histological features of terminal bronchiole and alveoli, (H&E stain, scale bar 100 µm, scale bar 20 µm),c and d: Normal histological appearance of respiratory bronchiole, alveolar duct and alveoli, (H&E stain, scale bar 100 µm, scale bar 20 µm).

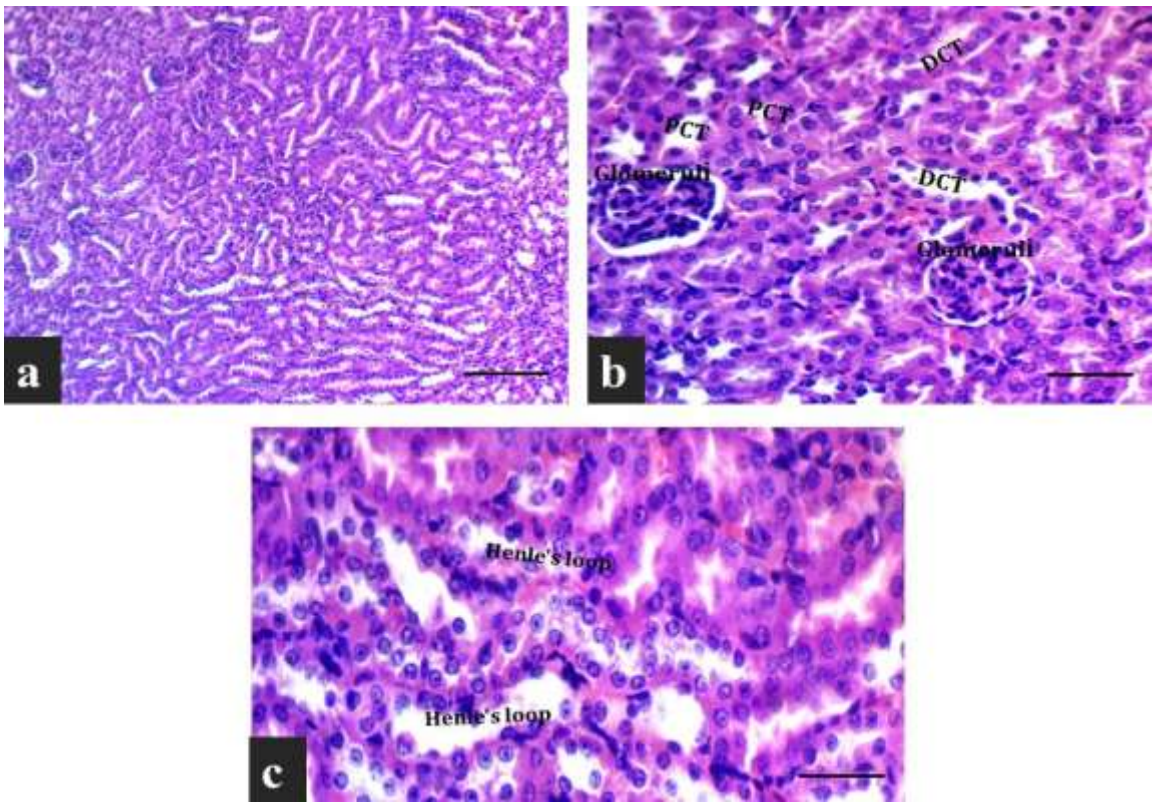

Figure 4.6: Microscopical section of kidney in control group G1. a: Normal histological features of renal parenchyma, b: Normal structures of glomeruli with distal (DCT) and proximal (PCT) convoluted tubules, c: Henle lubes showed normal histological appearance,(H&E stain, scale bar 100 µm, scale bar 20 µm).

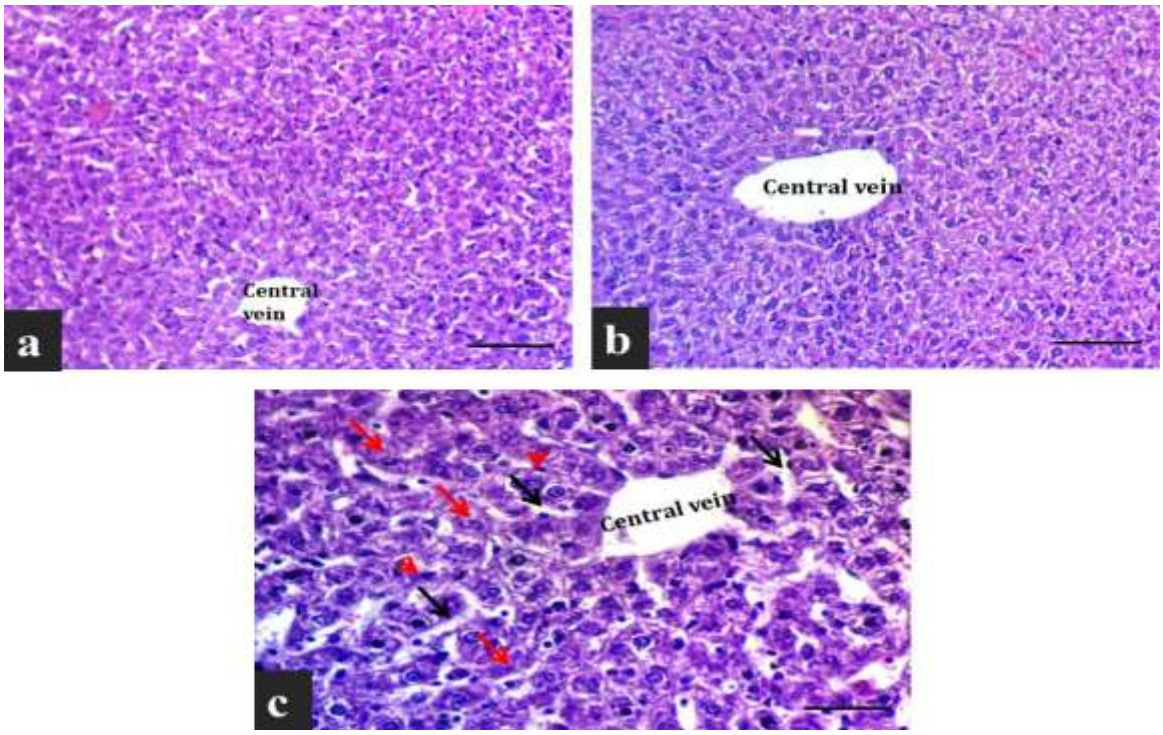

Figure 4.7:- Microscopical section of liver in control group G1. a-c: The central vein, rows of hepatocyte (red arrows) with sinusoidal capillaries (black arrows), also kuffer cells (head arrows) show normal histological feature, (H&E stain, scale bar 100 µm, scale bar 50 µm, scale bar 20 µm).

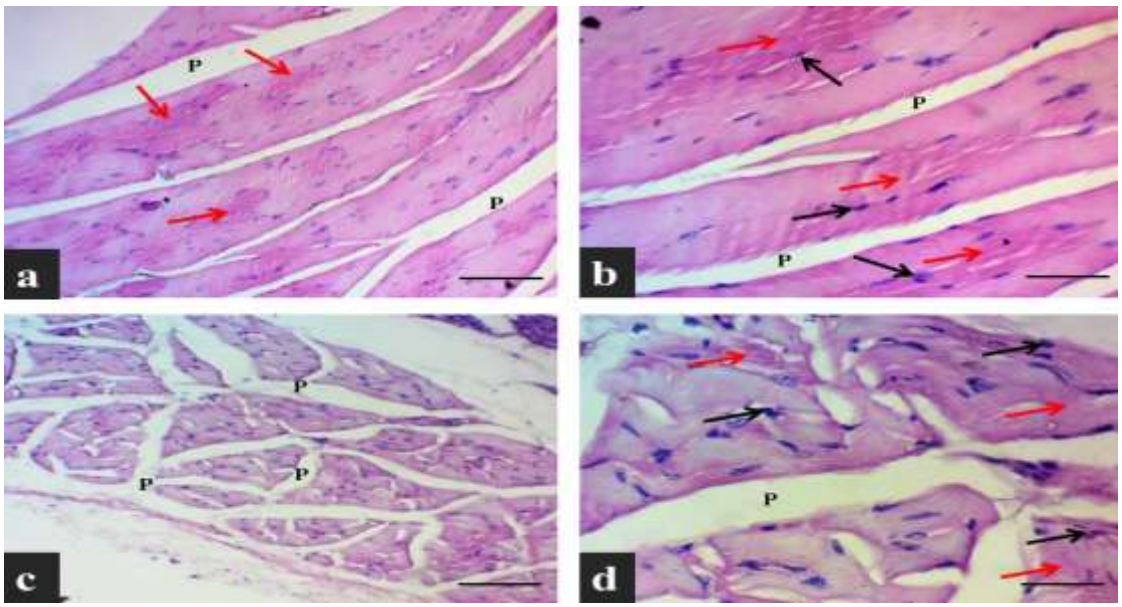

Figure 4.8:- Microscopical section of the skeletal muscle of cisplatin group G2. In longitudinal (a and b), and transverse (c and d) sections showed severe degeneration/necrosis including, the histological features of muscle fiber cells showed collapsing of muscle fibers in transverse sections, small focal or massive necrosis appeared by eosinophilic stained (red arrows) and pyknotic nuclei (black arrows), perimysium space was significantly expanded (P), (H&E stain, scale bar 100 µm, scale bar 20 µm).

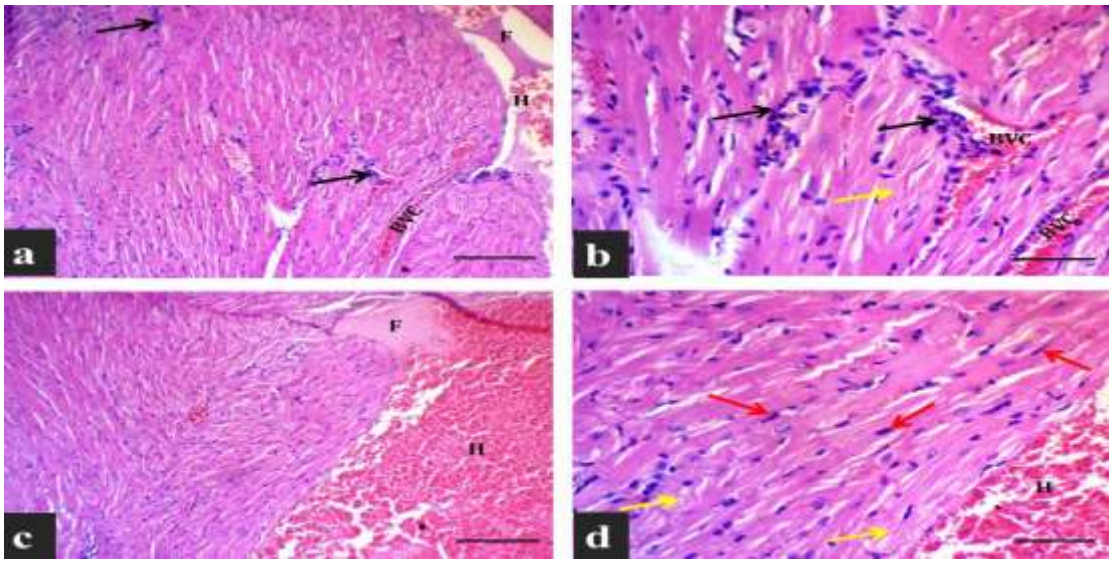

Figure 4.9:- Microscopical section of the cardiac muscle of G2 cisplatin group. a-d: cardiac muscle fiber were distorted markedly, focal deposition of fibrin (F), focal peri-vascular infiltration of neutrophils (black arrows), congestion of blood vessels (H), disruption in continuity of individual muscle fibers with focal lysis of sarcoplasm (yellow arrows), necrotic myocyte with pyknotic nuclei as indicated by red arrows, (H&E stain, scale bar 100 µm, scale bar 20 µm).

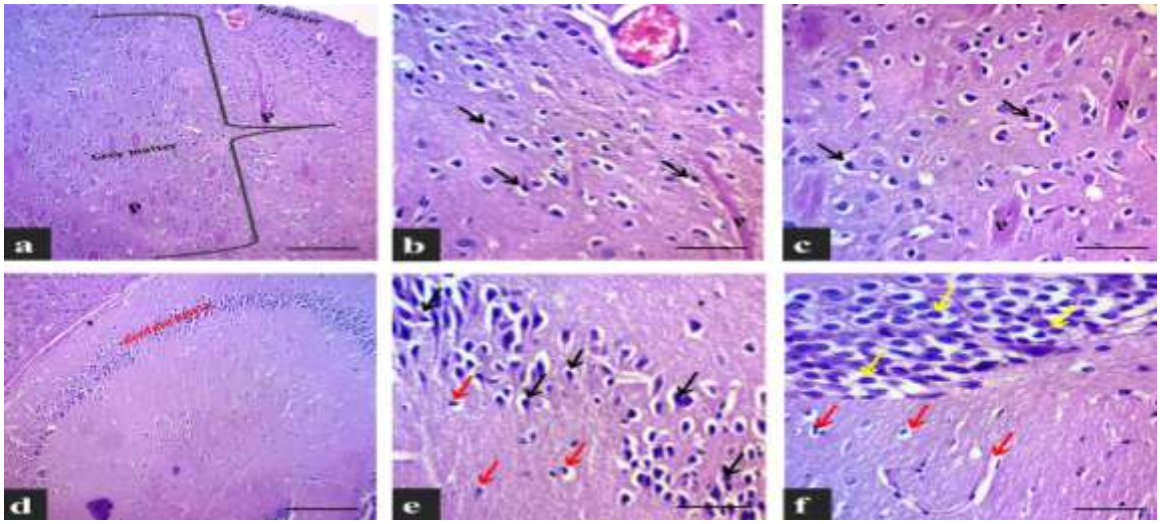

Figure 4.10:- Microscopical section of the brain of G2 cisplatin group. a: cerebrum tissue showed severely reduced number of neuronal and glial cells with aggregation of multiple-focal eosinophilic plaques (P), (H&E stain, scale bar 100 µm), b and c: Cerebrum sections showed vascular congestion, highly vacuolated neuron and glial cells, black arrows were showing the pyknotic nuclei and presence of focal eosinophilic plaques (P), (H&E stain, scale bar 20 µm), d-f: Hippocampus section had shown markedly losing in cell arrangement that exhibited edematous and vacuolated tissue architecture, markedly loss of neuronal, glial, and cortical cells, the black arrows (neurons), glial (red arrows), and yellow arrows (cortical cells) were showing the shrinkage, and nuclear condensation or pyknotic nuclei, (H&E stain, scale bar 100 µm, scale bar 20 µm).

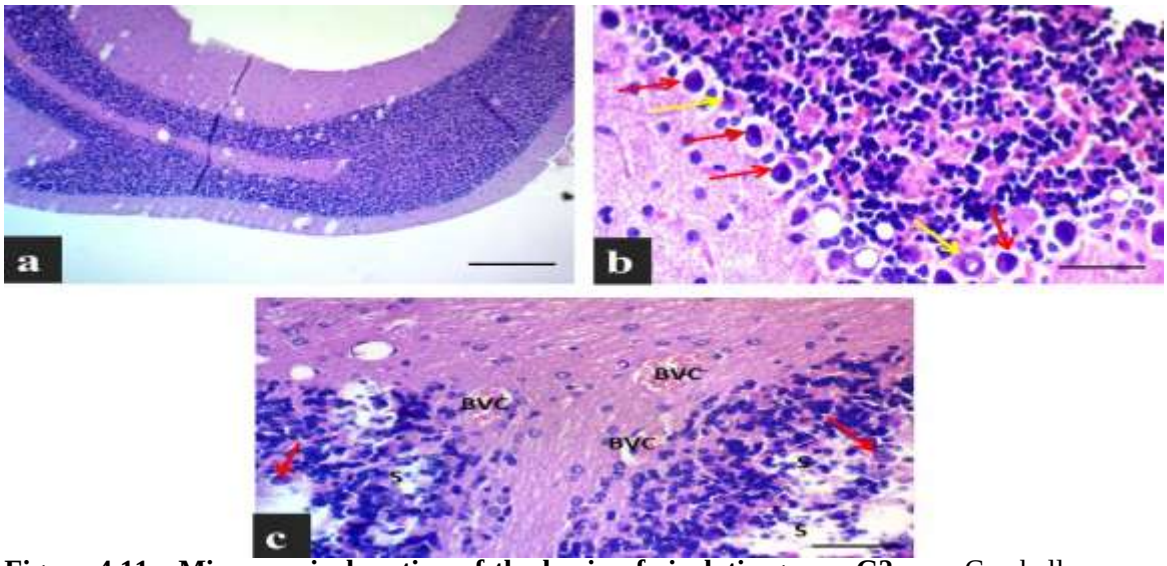

Figure 4.11:- Microscopical section of the brain of cisplatin group G2. a-c: Cerebellum showed neuronal spongiosis (S), blood vessels congestion (BVC), with apparent vacuolar space and disorganization in cerebellum layers, markedly loss of cells with pyknotic nuclei (red arrows), and some cells showed karyolitic nuclei as indicated by yellow arrows, (H&E stain, scale bar 100 µm, scale bar 20 µm).

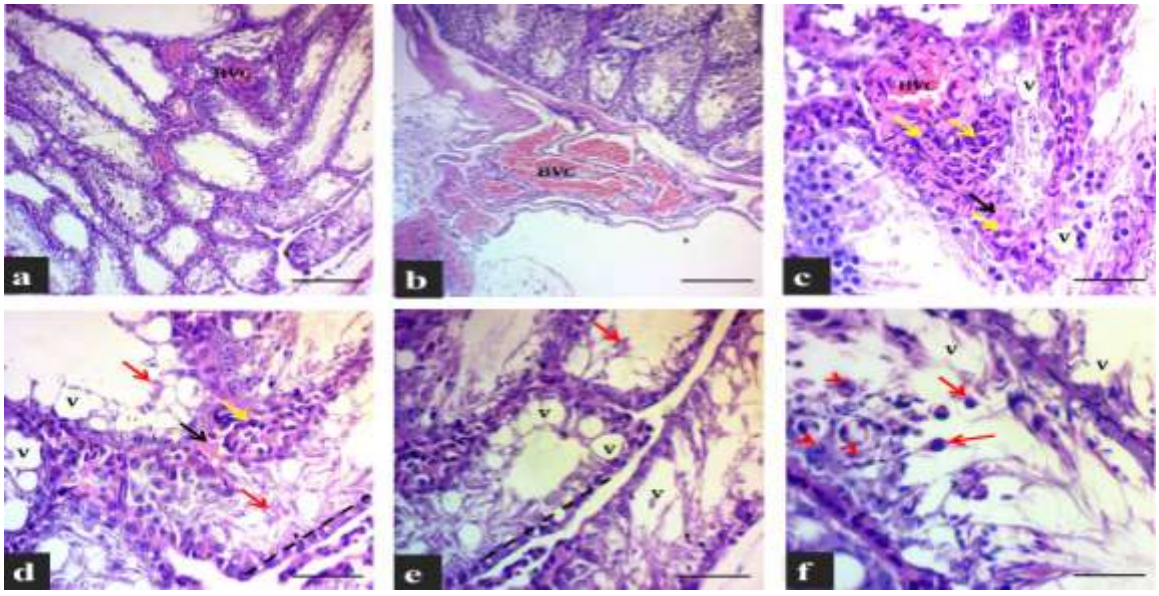

Figure 4.12:- Microscopical section of the seminiferous tubules of testis of G2 cisplatin group. a-e: revealed atypical morphological features; A massive degeneration in the seminiferous tubules, a disorganization of the germinal epithelium, tubules showed severe atrophy as they were devoid of epithelium with extensive loss of the spermatogenic cells specially spermatocytes, spermatids and exfoliation of the germ cells (red arrows), with only sertoli cells and spermatogonia (dash line) present within the depleted tubules, marked interstitial edema, congestion in blood vessels with in tubules and in the tunica albugina in section b, and replacement of the interstitial stroma with homogeneous eosinophilic material (black arrows), and depletion of leydig cells, focal infiltration of inflammatory cells (yellow arrows), hydropic degeneration or vaculation of spermatogenic and leydig cells (V), (H&E stain, scale bar 100 µm, scale bar 20 µm). f: Marked necrosis of spermatogenic cells and presence of multinucleated cells as indicated by head arrows. (H&E stain, scale bar 20 µm).

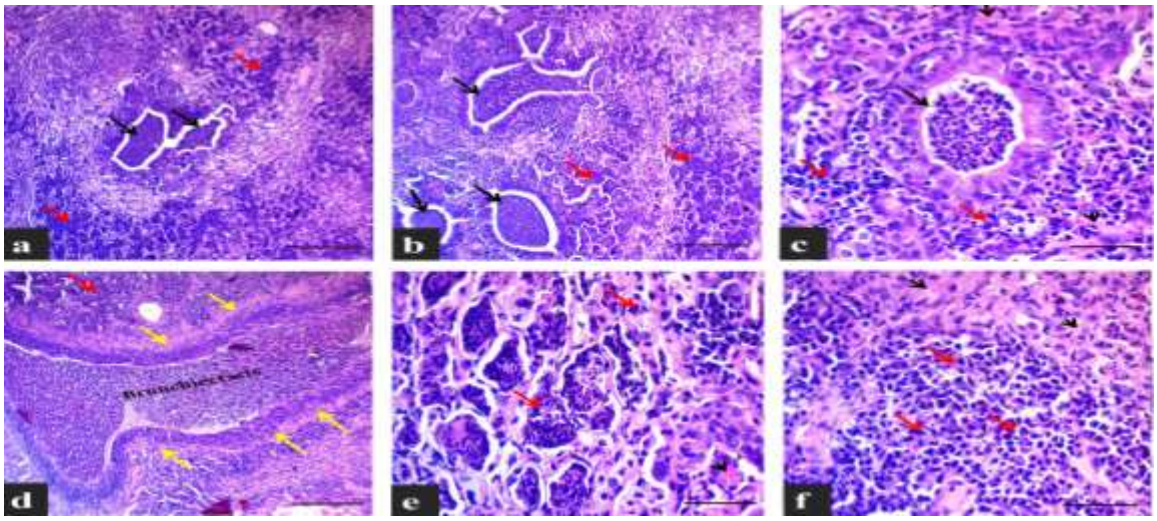

Figure 4.13:- Microscopical section of the lung tissue in G2 cisplatin group showed sappurative bronco pneumonia. a-c: The bronchi, bronchiole (black arrows) and alveoli (red arrows) are markedly distorted and filled with a massive purulent exudate with mononuclear inflammatory cells, d: The presence of bronchiectasis with mixture of neutrophils plus mononuclear inflammatory cells and mucus in the bronco-alveolar space also peri bronchiolar fibrosis (yellow arrows) were observed. e and f: The alveolar lumen (red arrows) were filled with purulent exudate with chronic inflammatory cells, (H&E stain, scale bar 100 µm, scale bar 20 µm).

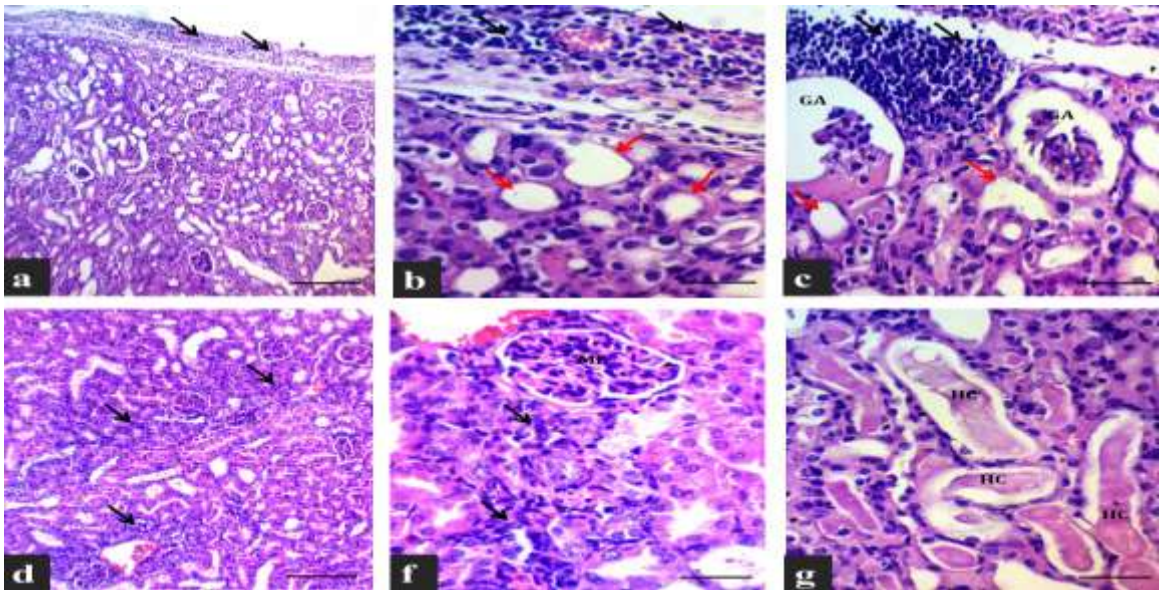

Figure 4.14:- Microscopical section of the kidney in G2 cisplatin group showed severe lesions. a-c: Marked inflammation (black arrows) in capsule +sub capsular regions and extend into the renal parenchyma with severe necrosis of proximal and distal convoluted tubules (red arrows) with completely brush borders sloughing, obvious hyaline cast in renal tubules, in section c, infiltration of mononuclear inflammatory cells within interstitial tissue with completely atrophied of glomeruli, (H&E stain, scale bar 100 µm, scale bar 20 µm), d and f: Diffuse infiltration of inflammatory cells in interstitial tissue with mildly increasing glomerular cellularity in the form of mesangial expansion (ME), vascular congestion, marked degeneration of collecting tubules, g: Severe degeneration of loops of Henle (H&E stain, scale bar 100 µm, scale bar 20 µm).

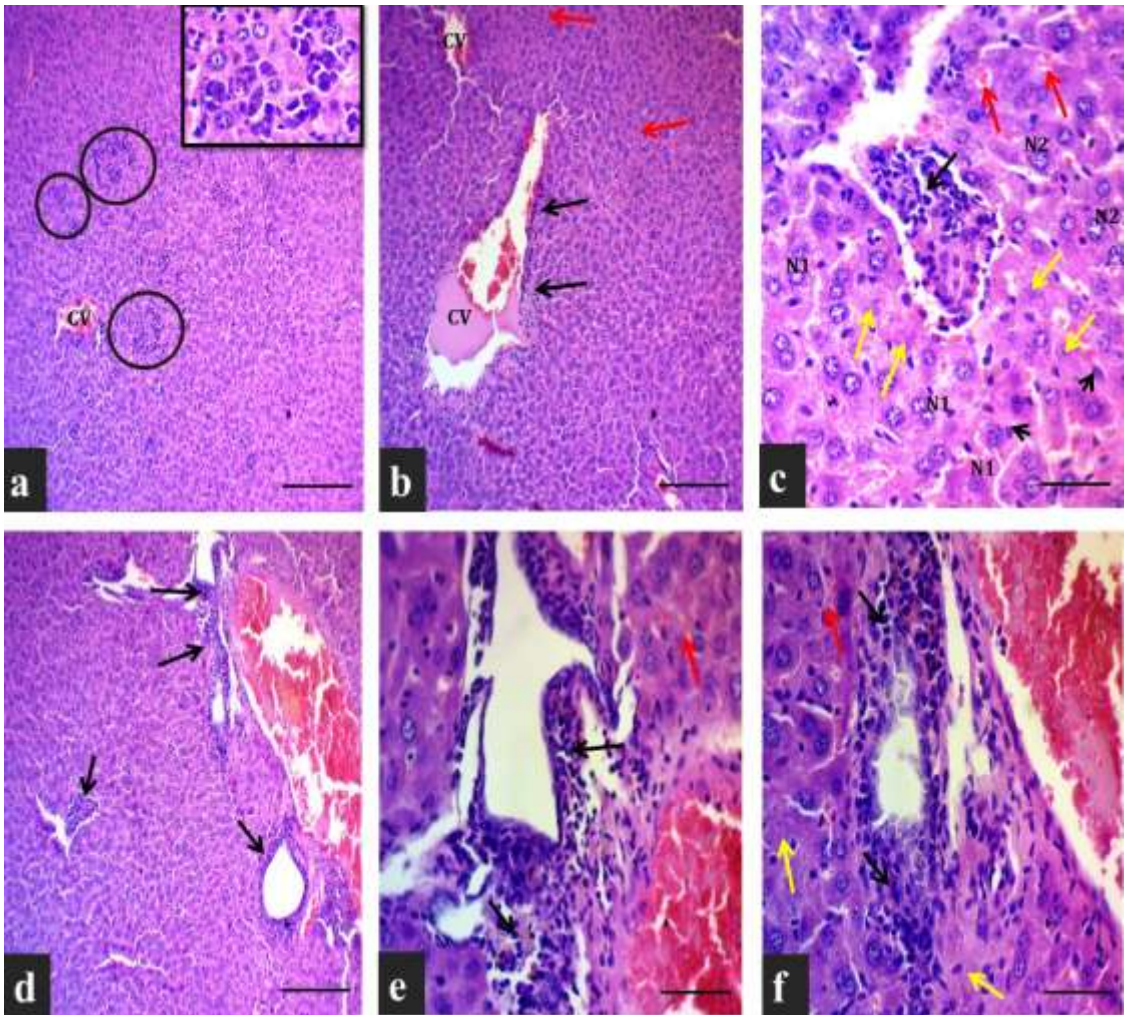

Figure 4.15:- Microscopical section of the liver in cisplatin group G2. a-c: Revealed an extensive degeneration/necrosis with inflammation; Marked central vein congestion (CV) with red blood cells pooling in the dilated sinusoids (red arrows), infiltration of polymorpho nuclear cells (black arrows) in the lumen of central vein in section b, in centrilobular region also localized microabcess (circle) involving a few hepatocytes with inflammatory cells and necrotic debris (insert), also with in the sinusoidal lumen, centrilobuar necrosis and some hepatocytes nuclei undergo karyolysis (yellow arrows), hepatocytes showed severely degeneration with different sized nuclei as large nuclei (N1), and small nuclei (N2) with activated kuffer cells as indicated by arrows head, (H&E stain, scale bar 100 µm, scale bar 20 µm). d-f: Congestion of portal vein, periportal infiltration of polymorpho nuclear cells (black arrows), also periportal hepatic necrosis as indicated by yellow arrows, (H&E stain, scale bar 100 µm, scale bar 20 µm).

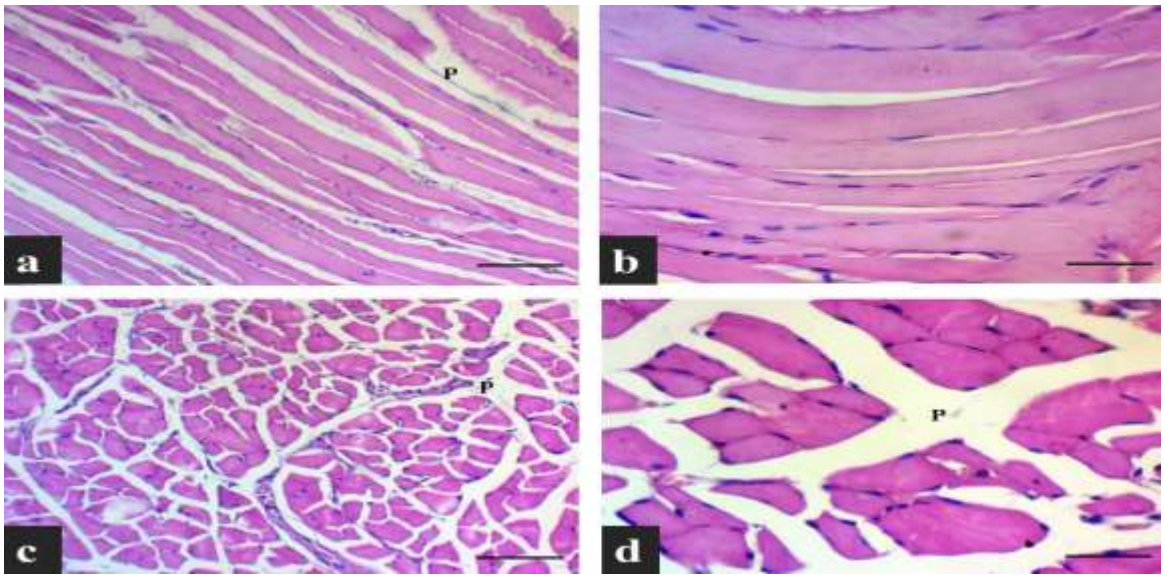

Figure 4.16:- Microscopical section of the skeletal muscle of 5mg/kg of memantine treated group G3. Histological features in longitudinal (a and b), and transverse (c and d) sections exhibited mild degeneration, Swollen of individual muscle fiber with intact sarcoplasm, nucleus, and myofilaments morphology, mild-moderate expansion of perimysium space (P), (H&E stain, scale bar 100 µm, scale bar 20 µm).

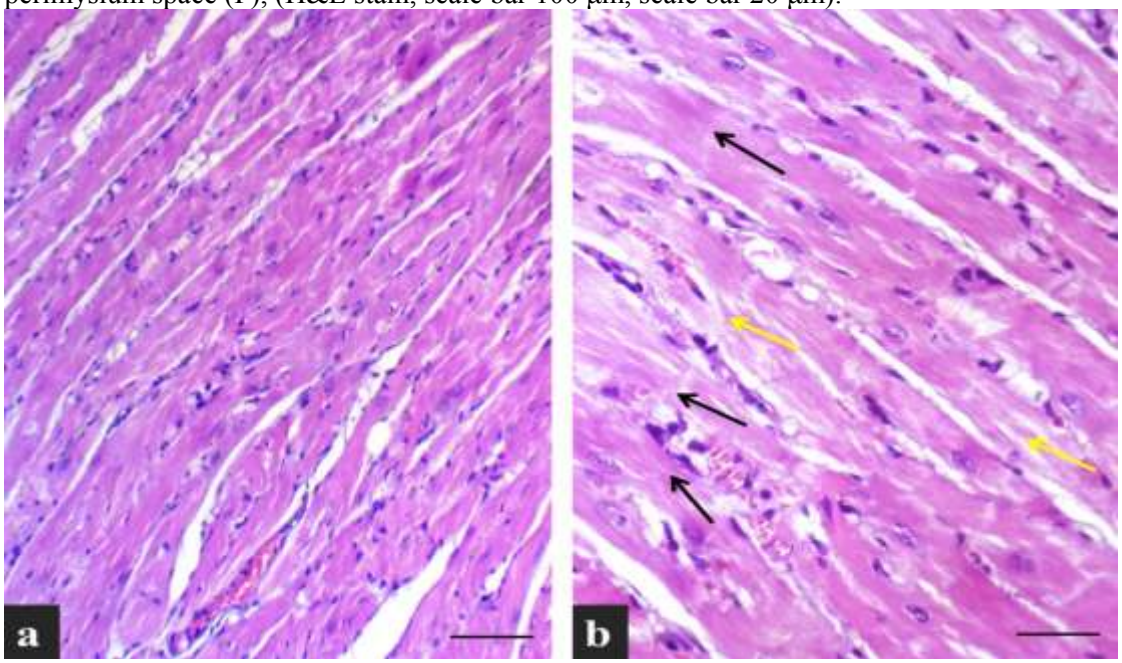

Figure 4.17:-. Microscopical section of the cardiac muscle of 5mg/kg of G3 memantine treated group. Histological features revealed mild degeneration, Swollen of individual myocyte fiber with intact sarcoplasm, nucleus, and striation features, focal damages (lysis) of myocytes (yellow arrow), intercalated disc remain intact as indicated by black arrows, (H&E stain, scale bar 100 µm, scale bar 20 µm).

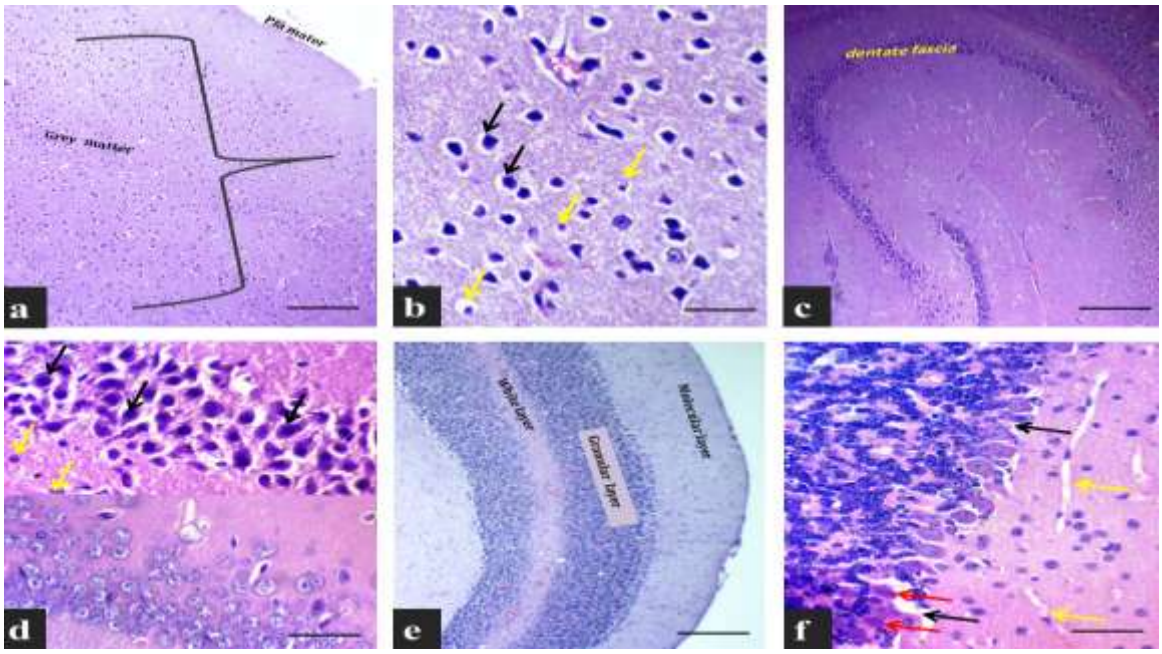

Figure 4.18:- Microscopical section of brain of 5mg/kg of G3 memantine treated group. a and b: Sections of cerebral tissue showed mild vacuolar spaces around the pyramidal cells (black arrows) and glial cells as indicated by yellow arrows, (H&E stain, scale bar 100 µm, scale bar 20 µm), c and d: Hippocampus section appeared mildly loss in cortical and neuronal cells with vacuolation in glial cells (yellow arrows), and neuronal cells (black arrows), (H&E stain, scale bar 100 µm, scale bar 20 µm), e and f: Cerebellum sections showed mild spongiosis (black arrow) with necrosis of purkenji cell as indicated by red arrows, (H&E stain, scale bar 100 µm, scale bar 20 µm).

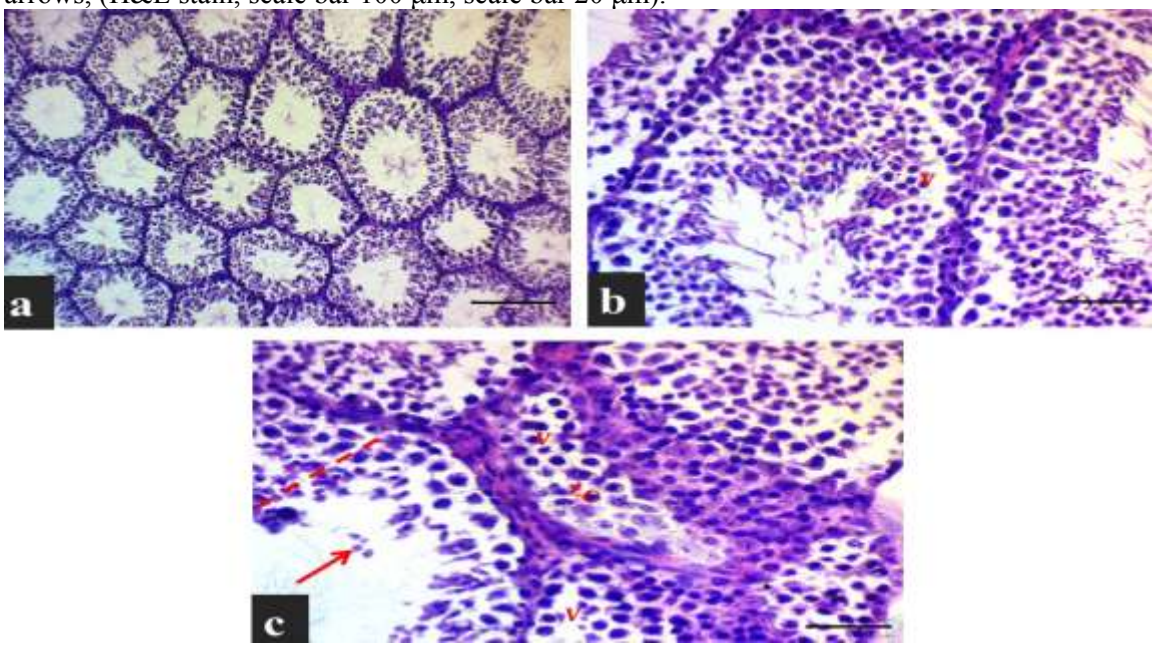

Figure 4.19:- Microscopical section of the seminiferous tubules of testis in 5mg/kg memantine treated group G3. a-c: Histological features revealed mild degeneration in the tubules, with mild loss of the spermatogenic cells (dash line), and exfoliation of the germ cells (red arrows), spermatogonia remain intact sperm mildly loosed, and mild vacuolation of leydig cells(V), (H&E stain, scale bar 100 µm, scale bar 20 µm).

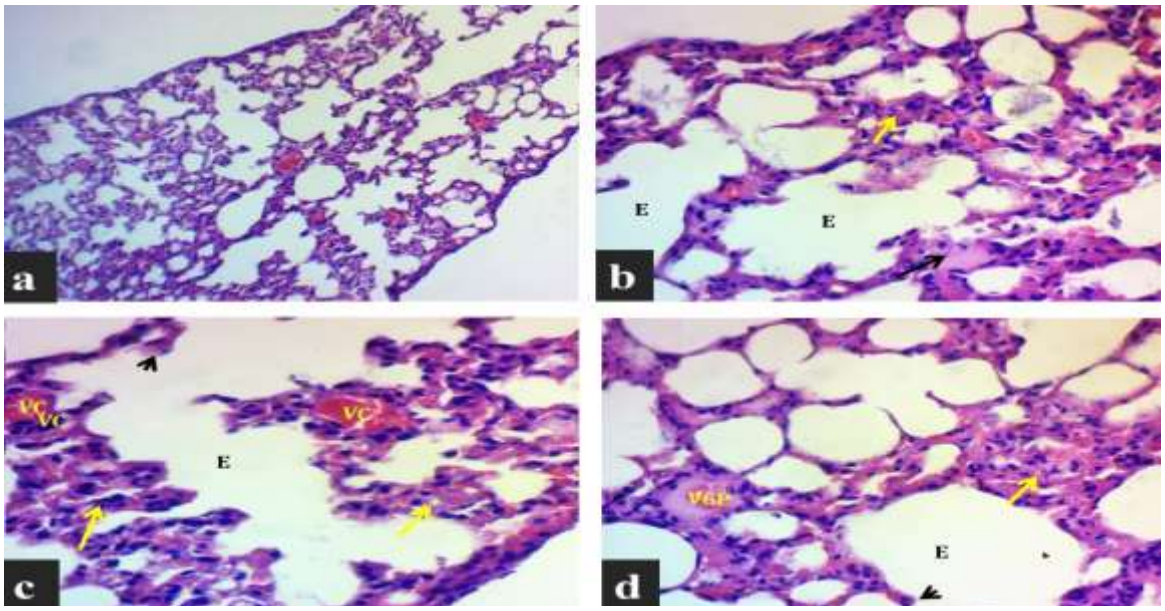

Figure 4.20:- Microscopical section of the lung tissue in 5mg/kg memantine treated group G3. a-d: Histological features revealed thickening of the alveolar septa due to congestion of the alveolar capillaries and infiltration of neutrophils (yellow arrows), small amounts of pinkish-stained protinacious fluid were seen within the alveolar spaces (black arrow), and congested blood vessels (CVP), marked distension of the pulmonary alveoli (E) with appearance of some fibrotic knobs (arrows head), (H&E stain, scale bar 100 μm, scale bar 20 μm).

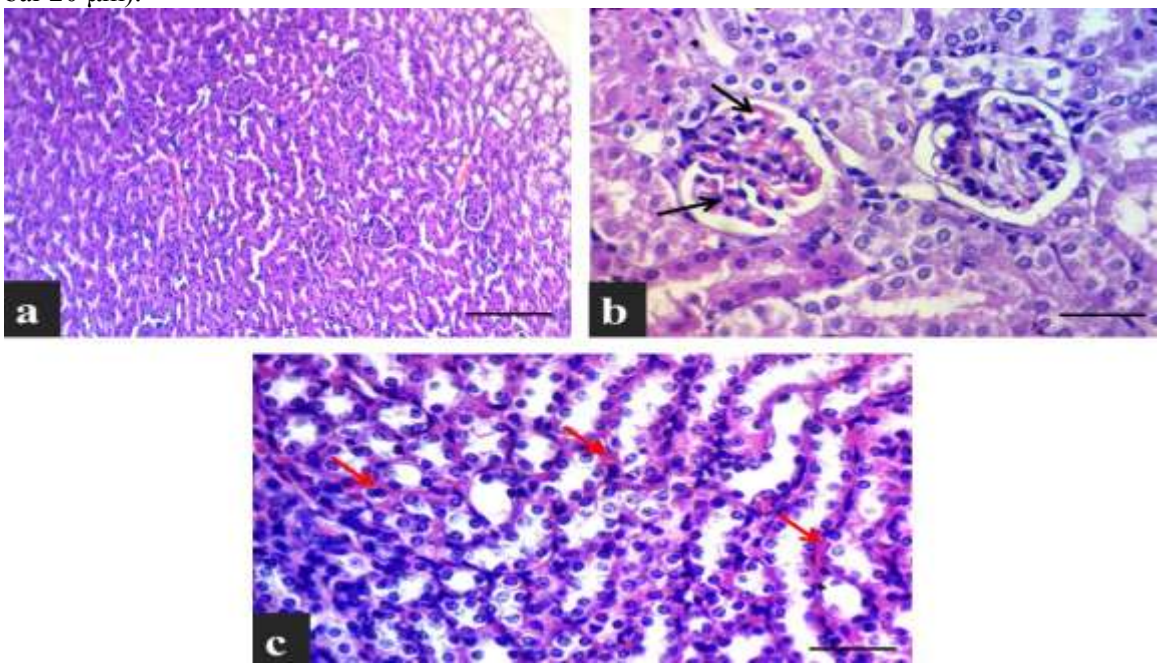

Figure 4.21:- Microscopical section of the kidney showed mild to moderate changes in G3 of 5 mg/kg of memantine. a and b: Mild hydropic degeneration of renal tubules with slightly blurring of brush borders, mild vascular congestion with interstitial hemorrhages (red arrows), the glomeruli showed mild dilation with congestion of mesangial capillary (black arrows). c: Mild- moderate degeneration of Henle loops with mild interstitial hemorrhages (red arrows), (H&E stain, scale bar 100 μm, scale bar 20 μm).

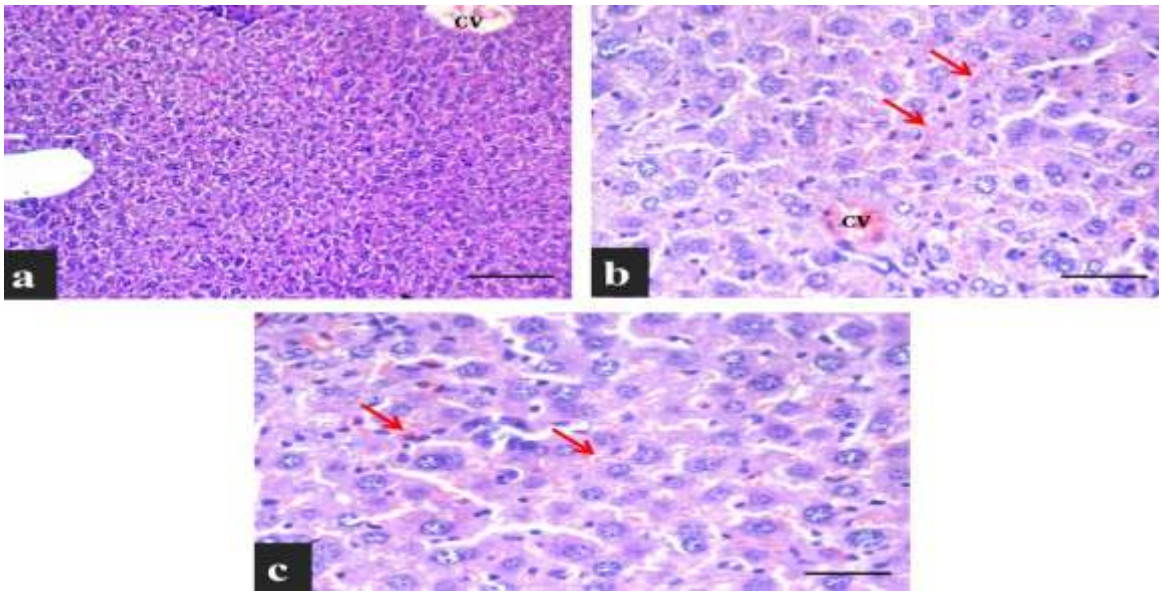

Figure 4.22:- Microscopical section of the liver in 5mg/kg of memantine treated group G3. a-c: liver section showed mild- moderate hydropic degeneration with centrally located nuclei, central vein congestion (CV) with RBCs pooling in the dilated sinusoids mildly (red arrows), infiltration of a few polymorpho nuclear cells (red arrows) with in the sinusoidal lumen, (H&E stain, scale bar 100 µm, scale bar 20 µm).

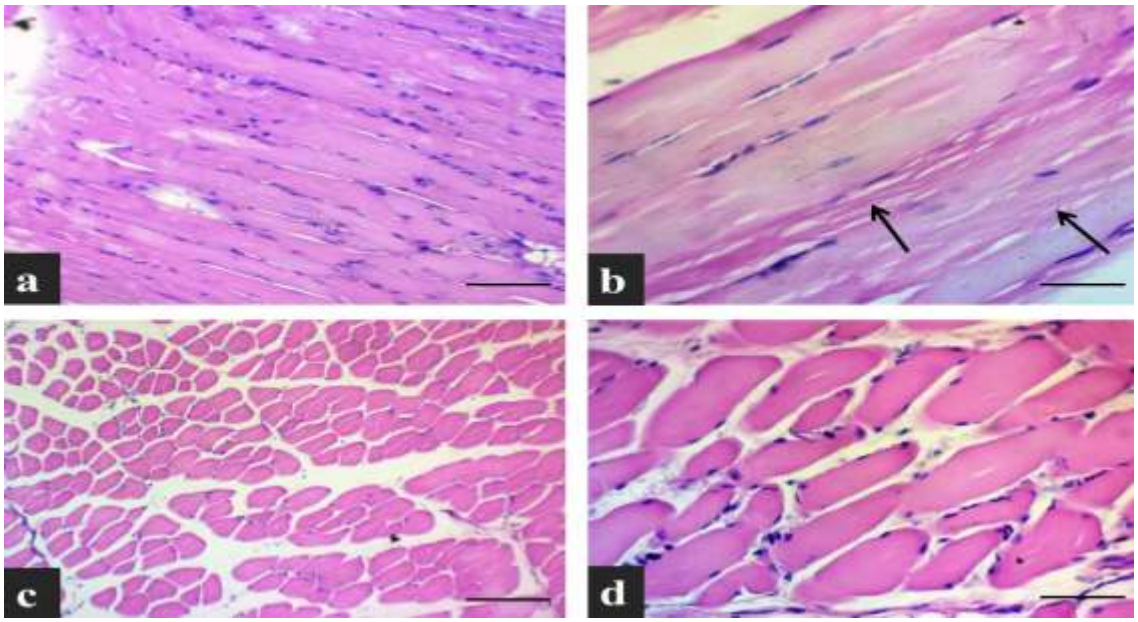

Figure 4.23:- Microscopical section of the skeletal muscle of cisplatin +5 mg/kg memantine group G4. In longitudinal (a and b), and transverse (c and d) sections showed moderate degeneration, muscle fiber showed a focal wavy sarcoplasm with focal collapsing of muscle fibers (black arrows) in longitudinal sections, moderate swelling of individual muscle fiber, perimysium space was moderately expanded (P), (H&E stain, scale bar 100 µm, scale bar 20 µm).

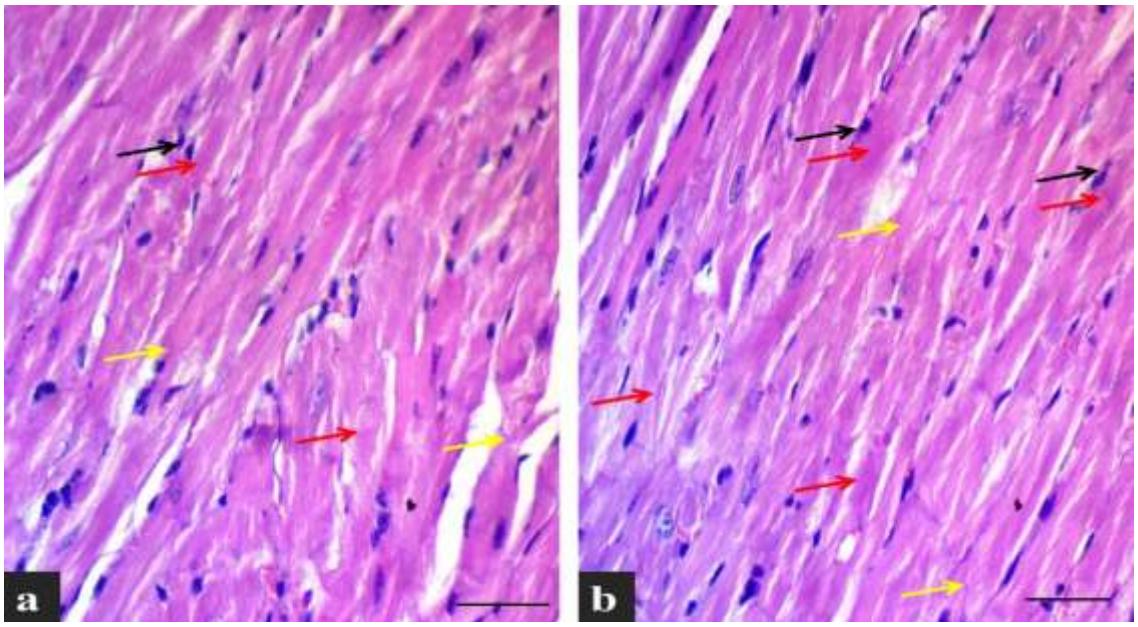

Figure 4.24:- Microscopical section of the cardiac muscle of cisplatin + 5 mg/kg memantine group G4. a and b: Moderate degeneration changes of myofibril structure; dissolving of fibers and striations with collapsed myocardial fibers, and small focal necrosis appeared by eosinophilic stained sarcoplasm (red arrows) with pyknotic nuclei (black arrows), moderate degeneration of intercalated disks as indicated by yellow and losing in continuity of cardiac muscles fiber, (H&E stain, scale bar 20 µm).

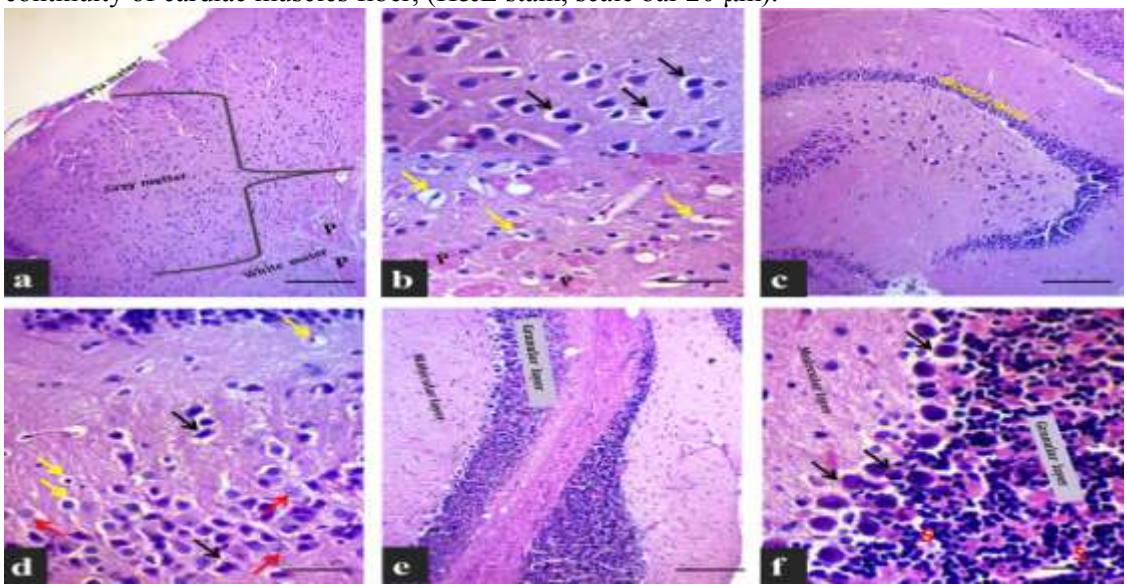

Figure 4.25:- Microscopical section of brain of cisplatin + 5mg/kg memantine group G4. a and b: cerebral tissue showed moderately losing of neuronal and glial cells with mildly aggregation of multiple focal eosinophilic plaques (P), moderately vacuolar spaces around the pyramidal cells (black arrows) and glial cells as indicated by yellow arrows, (H&E stain, scale bar 100 µm, scale bar 20 µm), c and d: Hippocampus section appeared modest loss in cortical and neuronal cells with vacuolation in glial cells (yellow arrows), and cortical cells (red arrows), the black arrows which were indication for neurons, (H&E stain, scale bar 50 µm, scale bar 20 µm), e and f: Cerebellum sections showed a modest loss in Purkinje's cells and minimized spongiosis with pyknotic nuclei as indicated by black arrows, (H&E stain, scale bar 100 µm, scale bar 20 µm).

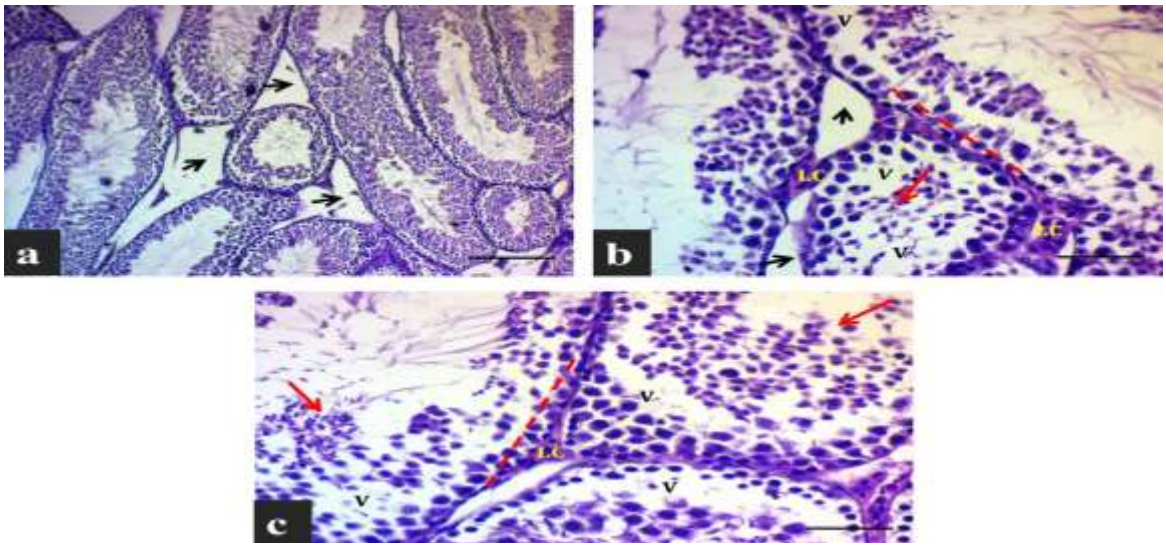

Figure 4.26:- Microscopical section of the seminiferous tubules of testis in cisplatin +5 mg/kg memantine group G4. a-c: Histological features revealed moderate degeneration/necrosis in the tubules, a disorganization of the germinal epithelium, with moderate loss of the spermatogenic cells and exfoliation of the germ cells (red arrows), spermatogonia remain intact (dash line), sperm mildly loosed, moderate interstitial edema (head arrows) with moderately depletion of leydig cells, and hydropic degeneration or vaculation of spermatogenic (V), (H&E stain, scale bar 100 μm, scale bar 20 μm).

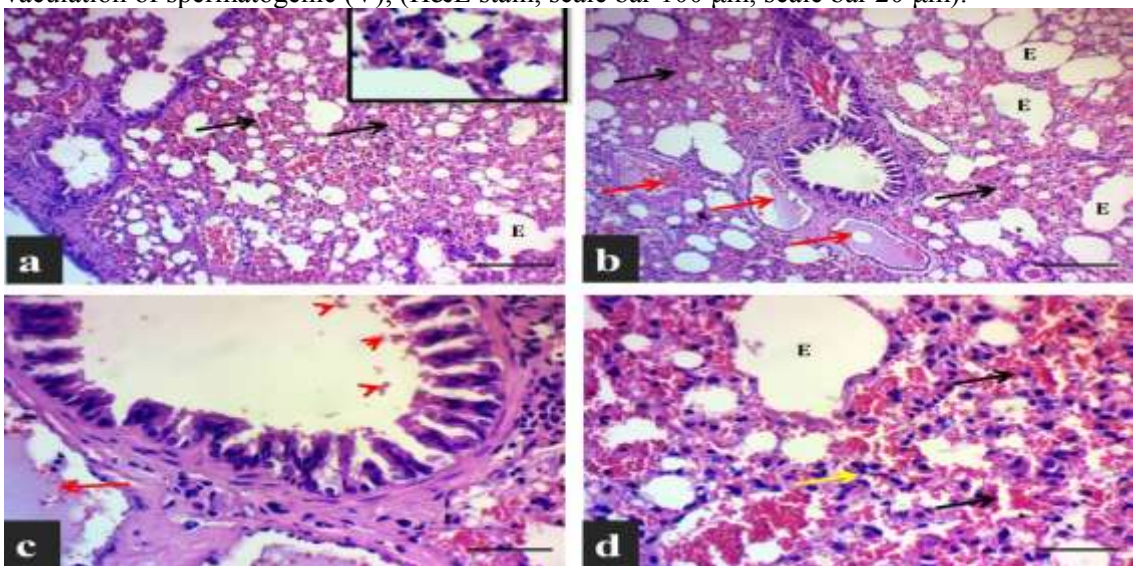

Figure 4.27:- Microscopical section of the lung parenchyma showed red stage of pneumonia in cisplatin + 5mg/kg memantine group G4. a-d: Histopathological lesion revealed moderate lesion; Moderate thickening of the alveolar septa due to congestion of the alveolar capillaries (insert) with inflammatory cells (neutrophil), vascular congestion with fibrinous exudate in their lumen (red arrows), extravasation of red blood cells into the alveolar spaces (black arrows) with focal numbers of neutrophils (yellow arrows), also extravasation of RBC in the bronchi lumen in section b with desquamation of lining epithelium and presence of necrotic debris in their lumen (arrows head), and destruction of some alveolar walls leads to emphysema (E), (H&E stain, scale bar 100 μm, scale bar 20 μm).

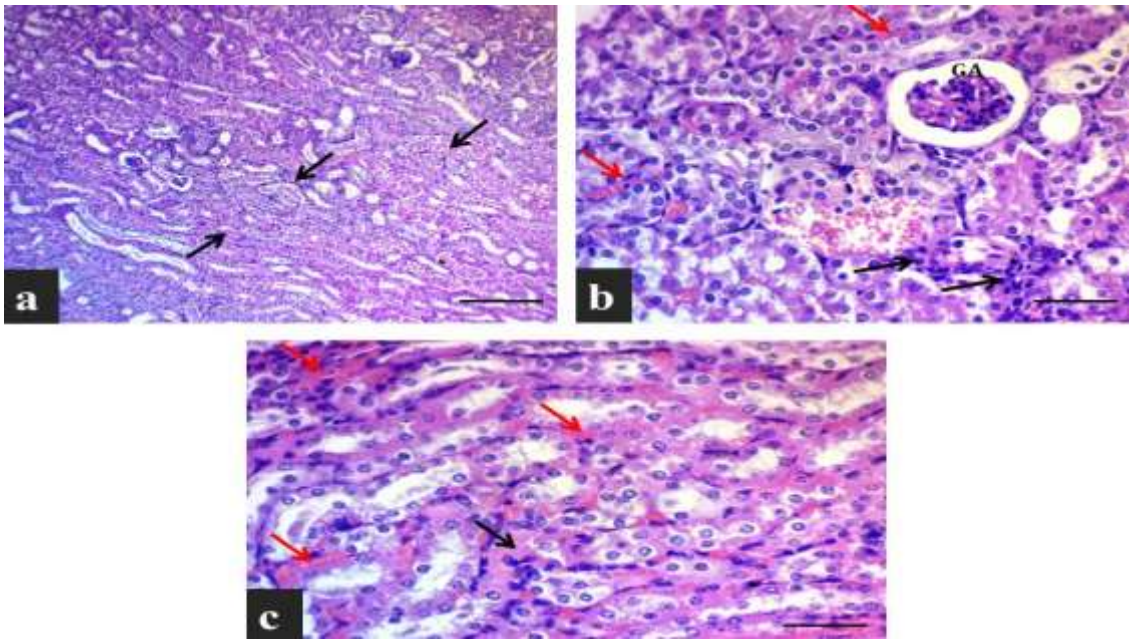

Figure 4.28:- Microscopical section of the kidney showed moderate changes in cisplatin + 5mg/kg memantine group G4. a and b: Moderate hydropic degeneration of renal tubules with blurring of brush borders, vascular congestion with interstitial hemorrhages, the glomeruli showed mild atrophy (GA) with dilation of Bowman's' capsule, and focal interstitial infiltration of neutrophils (black arrows). c: Moderate hydropic degeneration of Henle loops with interstitial hemorrhages (red arrows), and presence of hyaline cast in their lumen as indicated by black arrows, (H&E stain, scale bar 100 µm, scale bar 20 µm).

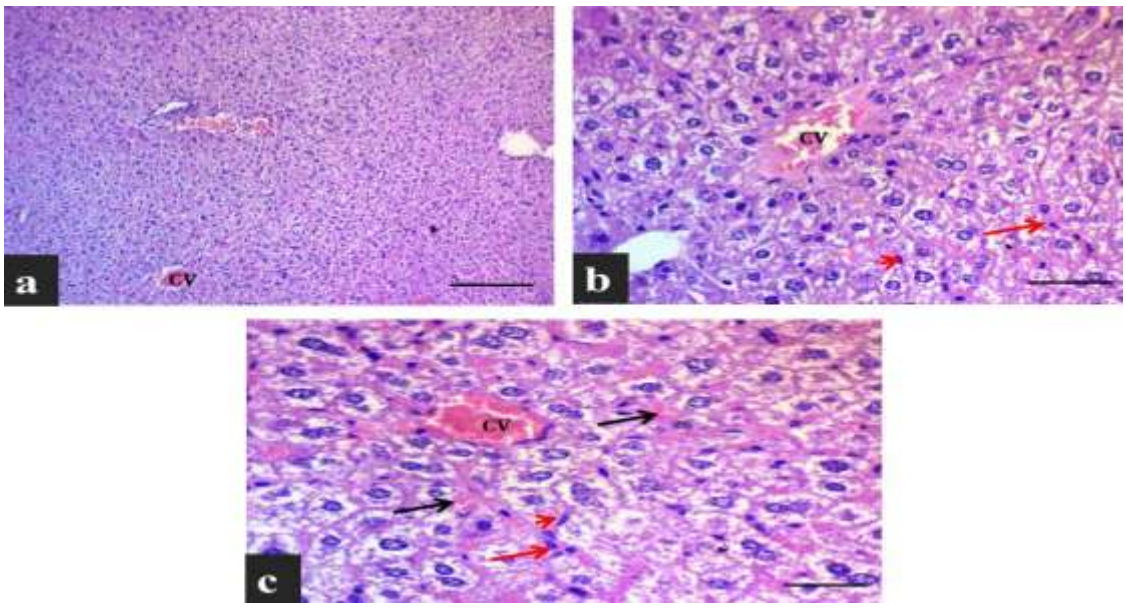

Figure 4.29:- Microscopical section of the liver in cisplatin + 5mg/kg memantine group G4. a-c: Histopathological liver section shown moderate degeneration; Central vein congestion (CV) with red blood cells pooling in the dilated sinusoids mildly (black arrows), infiltration of few polymorpho nuclear cells (red arrows) with in the sinusoidal lumen, hepatocytes showed hydropic degeneration; hepatocytes appeared wispy cleared and distending cytoplasm with accentually located nuclei, and mildly activation of kuffer cells as indicated by head arrows, (H&E stain, scale bar 100 µm, scale bar 20 µm),

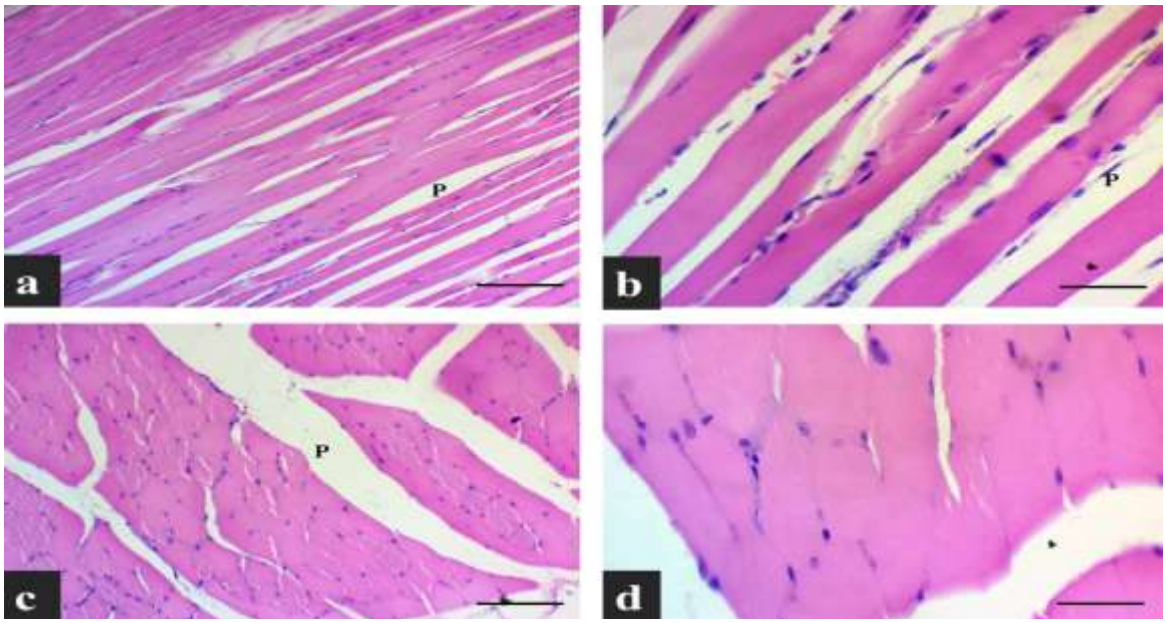

Figure 4.30:- Microscopical section of the skeletal muscle of cisplatin and 10 mg/kg of memantine treated group G5. Histological architecture in longitudinal (a and b), and transverse (c and d) sections showed mild degeneration by swollen of each individual muscle fiber with intact sarcoplasm, nucleus, and myofilaments structures, perimysium space mildly expanded (P), (H&E stain, scale bar 100 μm, scale bar 20 μm).

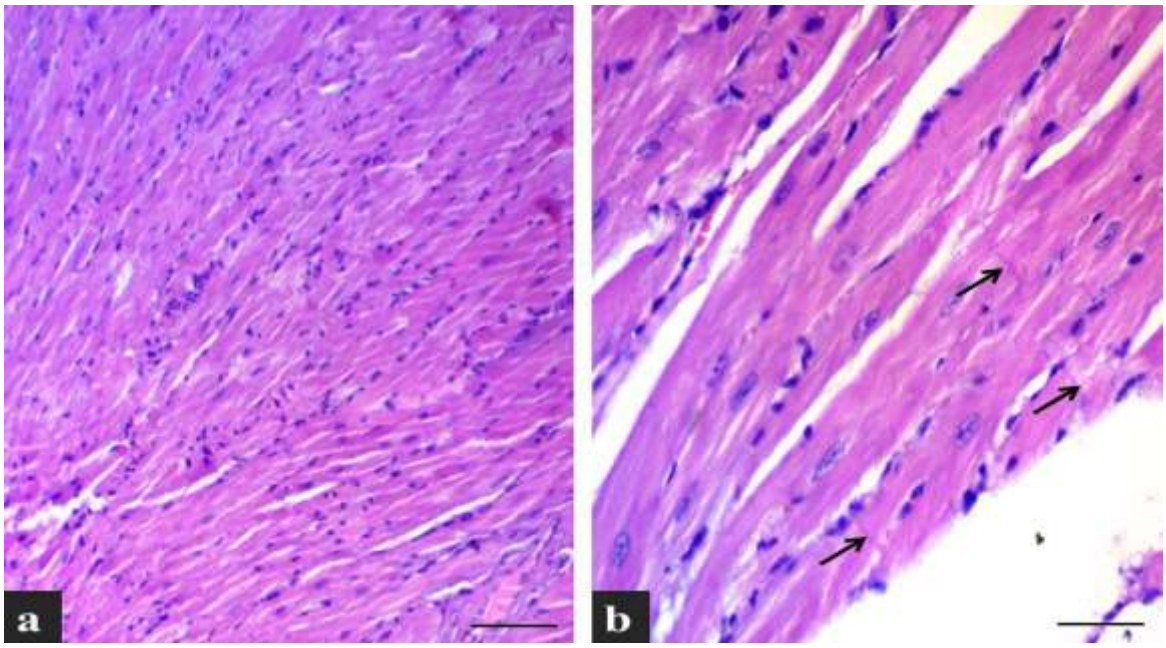

Figure 4.31:- Microscopical section of the cardiac muscle in cisplatin and 10mg/kg of memantine group G5. a an b: The myocardium show mild degeneration of myositis with the intact histological structure of myofibril and intercalated disc and normal arrangement or continuity of cardiac fibers without the evident of vascular abnormalities, (H&E stain, scale bar 100 μm, scale bar 20 μm).

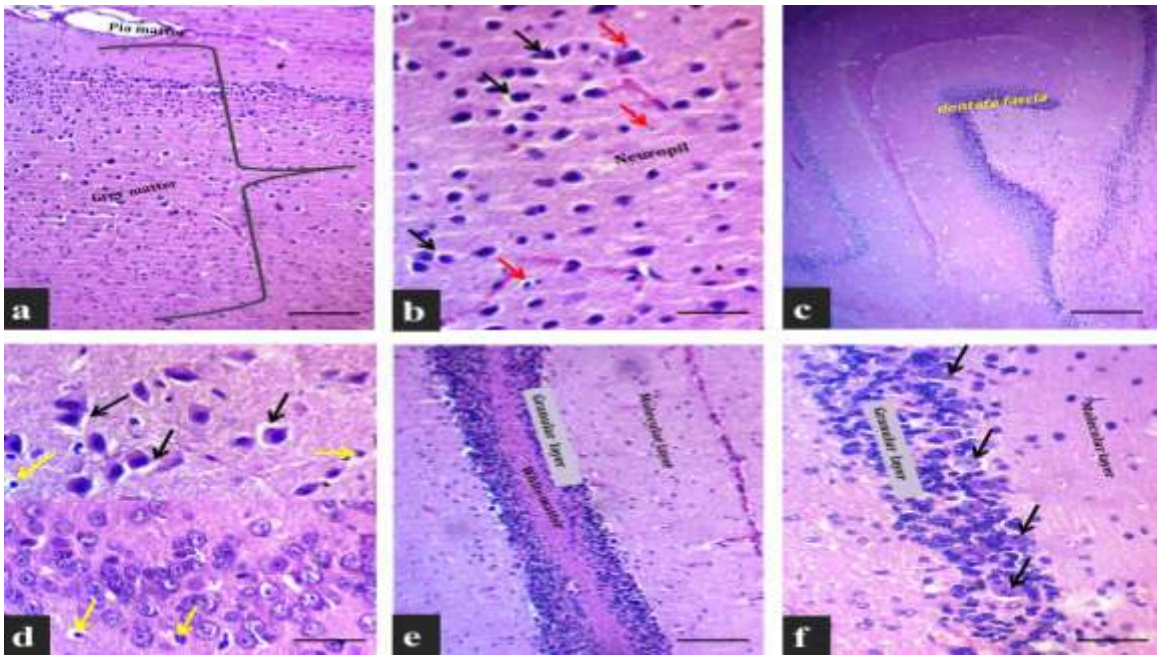

Figure 4.32:- Microscopical section of the brain of cisplatin and 10 mg/kg of memantine treated group G5. a and b: The cerebrum show mild degeneration in tissue architectures with slightly vaculation of neurons (black arrows), and glial cells as indicated by red arrows, (H&E stain, scale bar 100 µm, scale bar 20 µm), c and d: The hippocampus sections showed slightly alterations in the histoarchitecture, the numbers of cell were not reduced but pyramidal neuron (black arrows), and glial cells as indicated by yellow arrows showed mildly vacuolated, (H&E stain, scale bar 100 µm, scale bar 20 µm), e and f: The cerebellar layers have normal histopathological appearance with intact purkenji cells morphology as indicated by black arrows, (H&E stain, scale bar 50 µm, scale bar 20 µm).

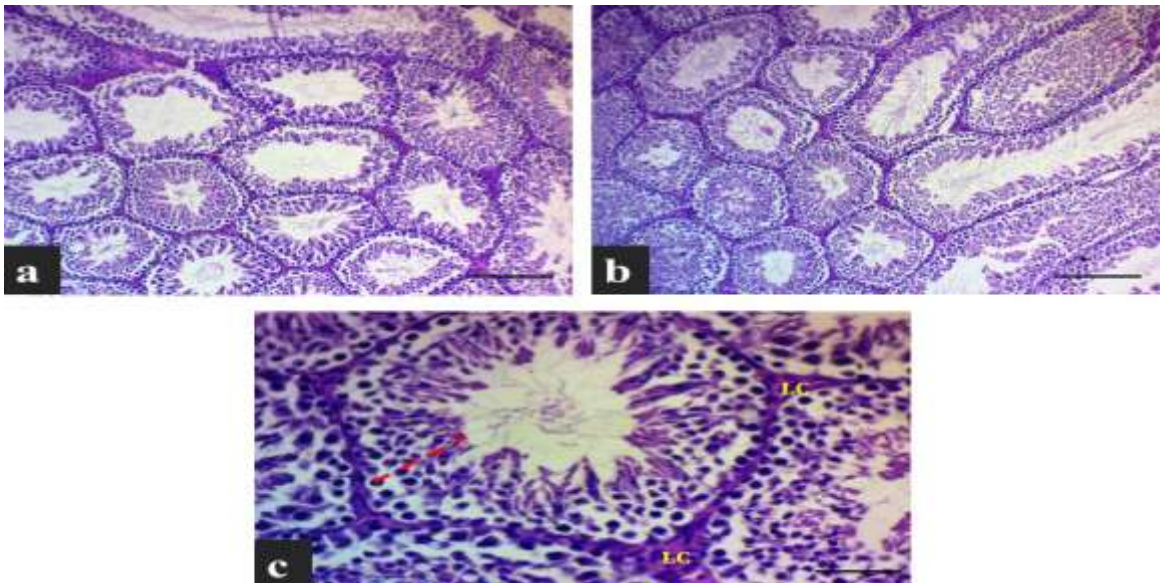

Figure 4.33:- Microscopical section of the seminiferous tubules of testis in cisplatin and 10 mg/kg of memantine treated group G5. a-c: The tubules showed mild degeneration and slightly disorganization of spermatogenesis series and loosing of spermatogenic cells (dash line) with germ and leydig cells (L) mildly, (H&E stain, scale bar 100 µm, scale bar 20 µm).

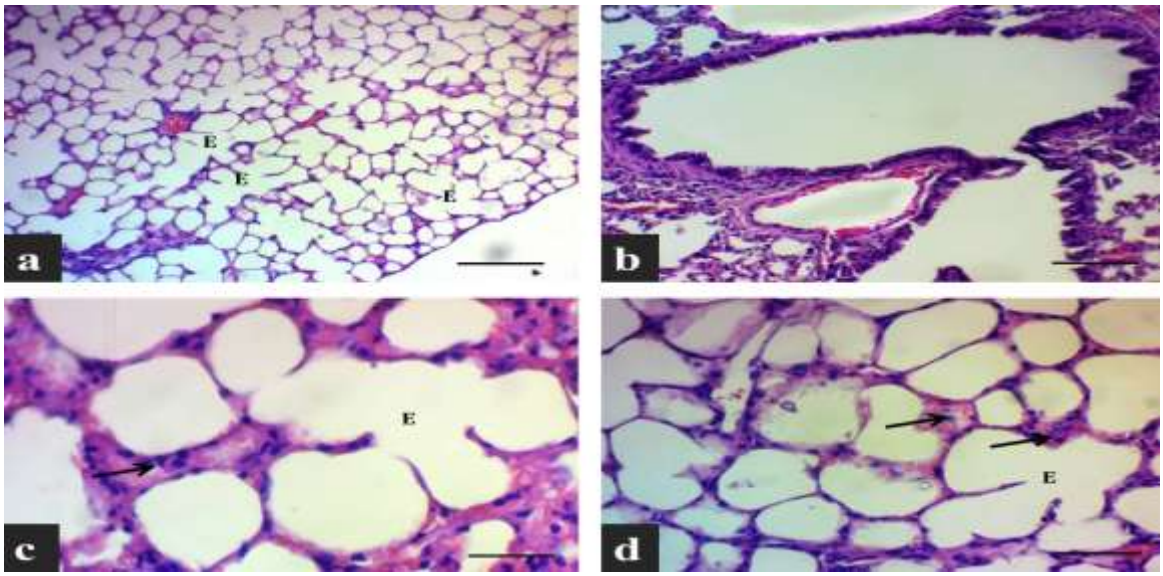

Figure 4.34:- Microscopical section of the lung parenchyma in cisplatin and 10mg/kg of memantine treated group G5. a-d: Histopathological lesion revealed; Focal-mildly thickening of the alveolar septa due to congestion of the alveolar capillaries with inflammatory cells (neutrophil) as indicated by black arrows, the bronchi epithelium remain intact in section b, and destruction of most alveolar walls leads to emphysema (E), (H&E stain, scale bar 100 µm, scale bar 20 µm).

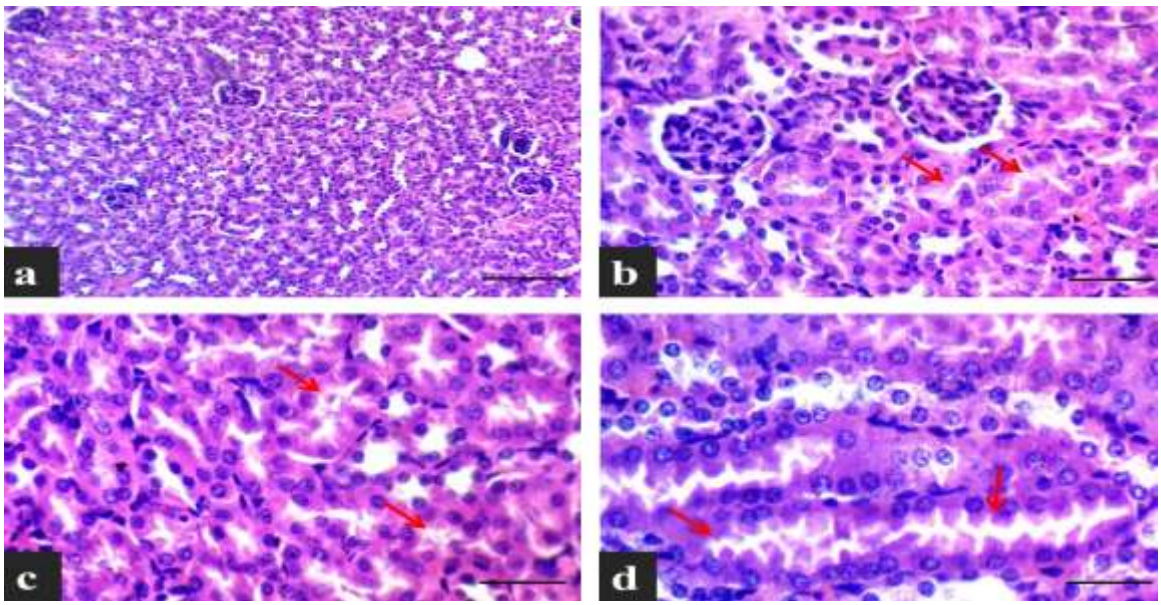

Figure 4.35:- Microscopical section of the kidney showed mild cell swelling in cisplatin and 10 mg/kg of memantine treated group G5. a-c: Mild swelling of renal tubules that forming star shaped appearance with slightly blurring of brush borders (red arrows), the glomeruli showed mild dilation of Bowman's' capsule. d: Mild swelling of Henle loops which appear as star liked structures (red arrows), (H&E stain, scale bar 100 µm, scale bar 20 µm).

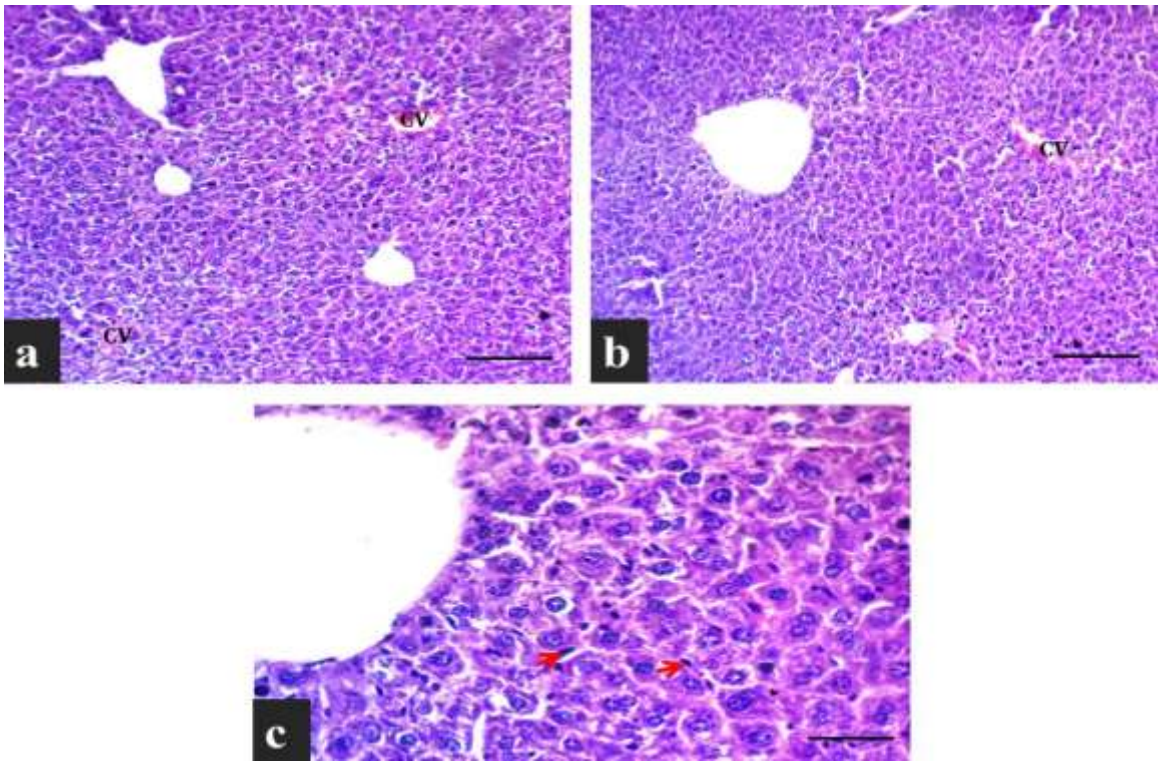

Figure 4.36:- Microscopical section of the liver in cisplatin and 10 mg/kg of memantine treated group G5. a-c: the histological features showed mild swelling of liver parenchyma; Dilation of central vein and few of them showed congestion, the sinusoidal capillary appeared normal architecture, hepatocytes mildly swelled with pale stained cytoplasm and centrally located nuclei, (H&E stain, scale bar 100 μm, scale bar 20 μm).

4.5. Immunohistochemical results of myeloperoxidase

Expression of myeloperoxidase in the renal tissues was evaluated semi-quantitatively by independent two pathologists. MPO staining was considered positive when there is brown cytoplasmic reactivity in mesangial cells in glomeruli, epithelial cells of distal and proximal convoluted tubules with Henle loops.

The expression scores were estimated as

No staining = 0 if no cells were stained with the myeloperoxidase marker,

1 = (weak) if the marker was expressed <25 % of the cells were stained,

2= (moderate) if expressed by 25%-50%, and

3= (strong) if it was expressed by 50% or more of the cells (Torrelo et al., 2015a)

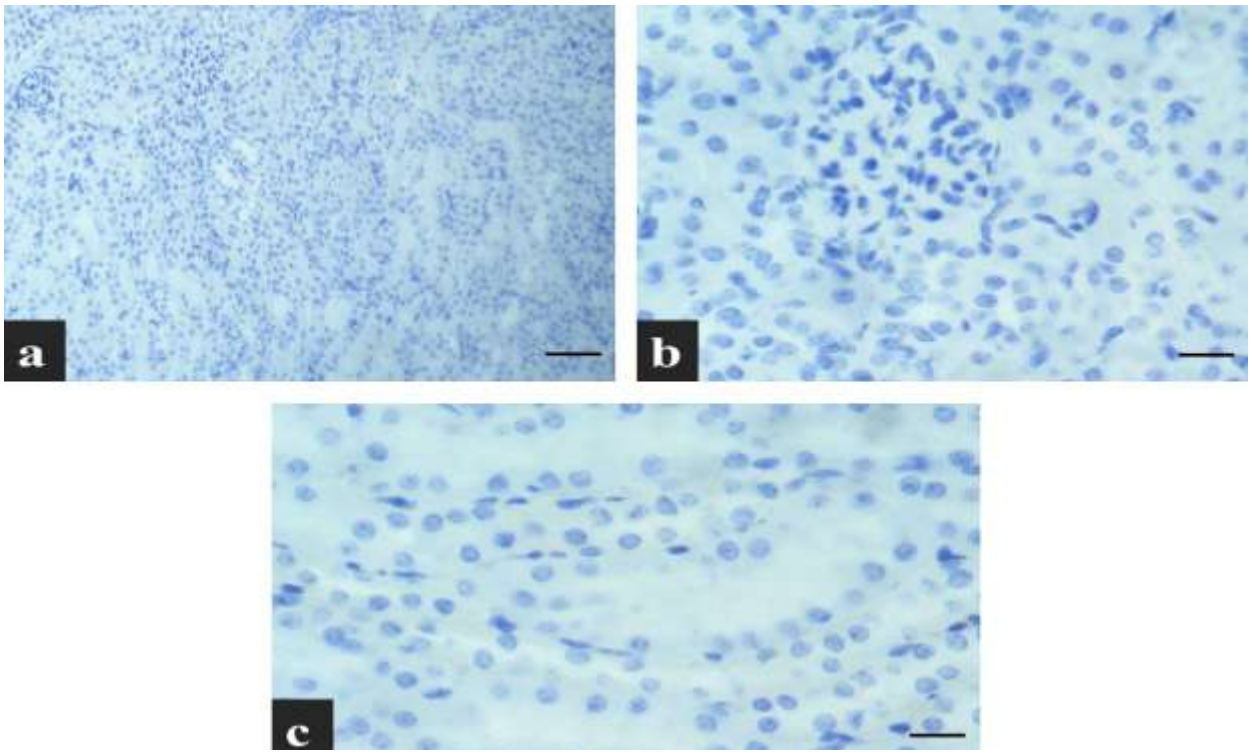

Figure 4.37: Expression of myeloperoxidase in kidney section in mice of control group G1. a-c: Score 0, no expression throughout the renal parenchyma, (scale bar 100μm, scale bar 20μm).

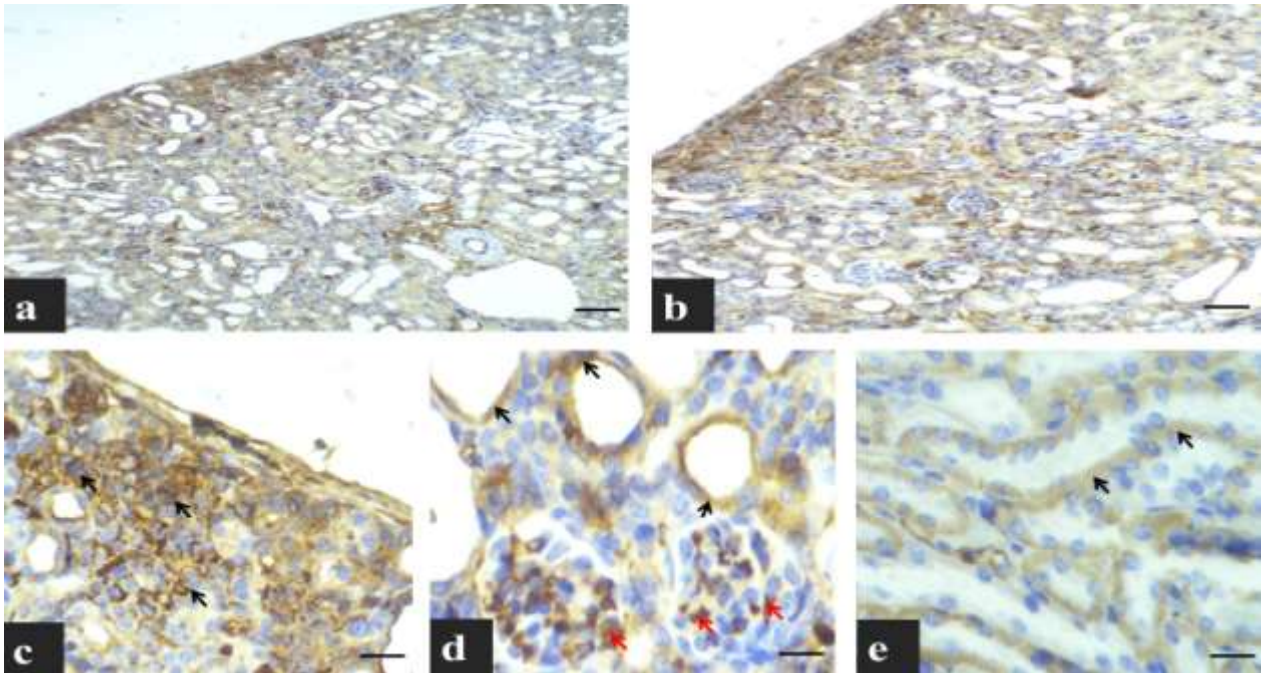

Figure 4.38: Expression of myeloperoxidase in kidney section of cisplatin group G2. a-e: strong cytoplasmic staining (score 3), in section c the arrows indicated expression of MPO in neutrophils, in section d the black arrows showed expression in proximal convoluted tubules, and the red arrows showed in mesangial cell expression of myeloperoxidase, in section e the arrows showed expression in Henle loops, (scale bar 100μm, scale bar 20μm).

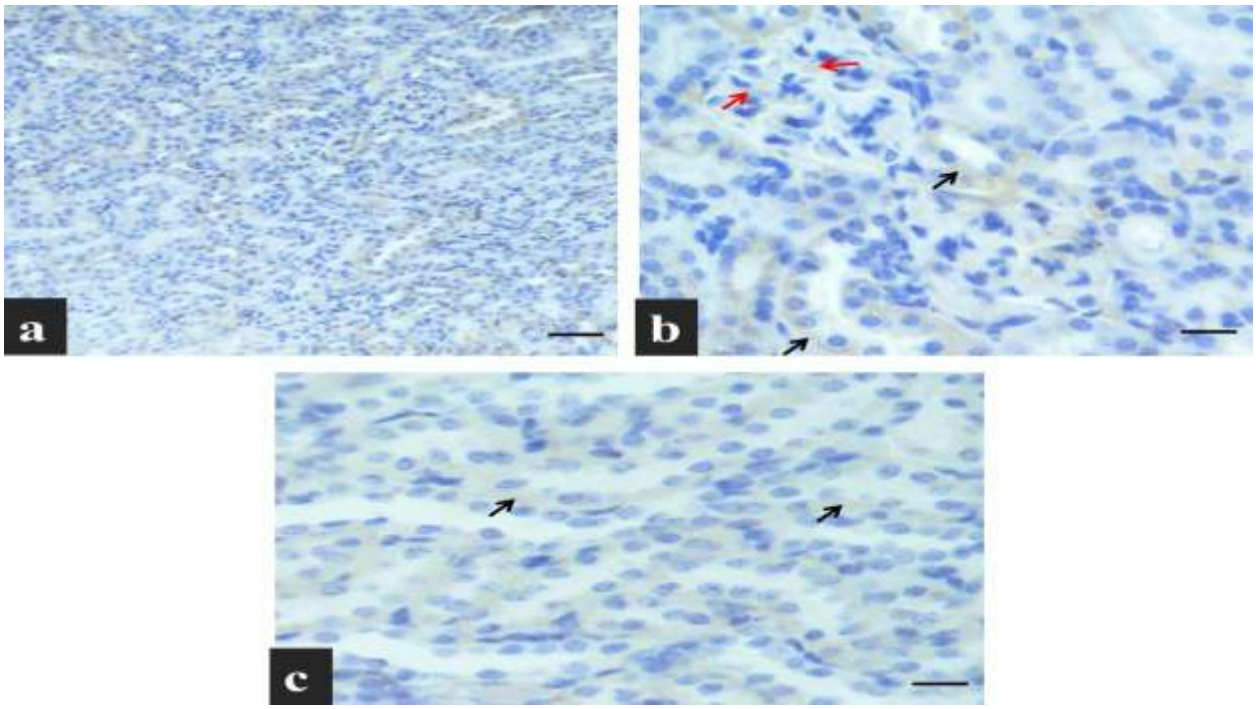

Figure 4.39: Expression of myeloperoxidase in kidney section in mice of 5mg/kg memantine treated group G3. a-c: Weak-local cytoplasmic staining (score 1), in section b the black arrows showed expression in proximal convoluted tubules, and the red arrows showed mesangial cell expression of MPO, in section c the arrows showed expression in Henle loops, (scale bar 100µm, scale bar 20µm).

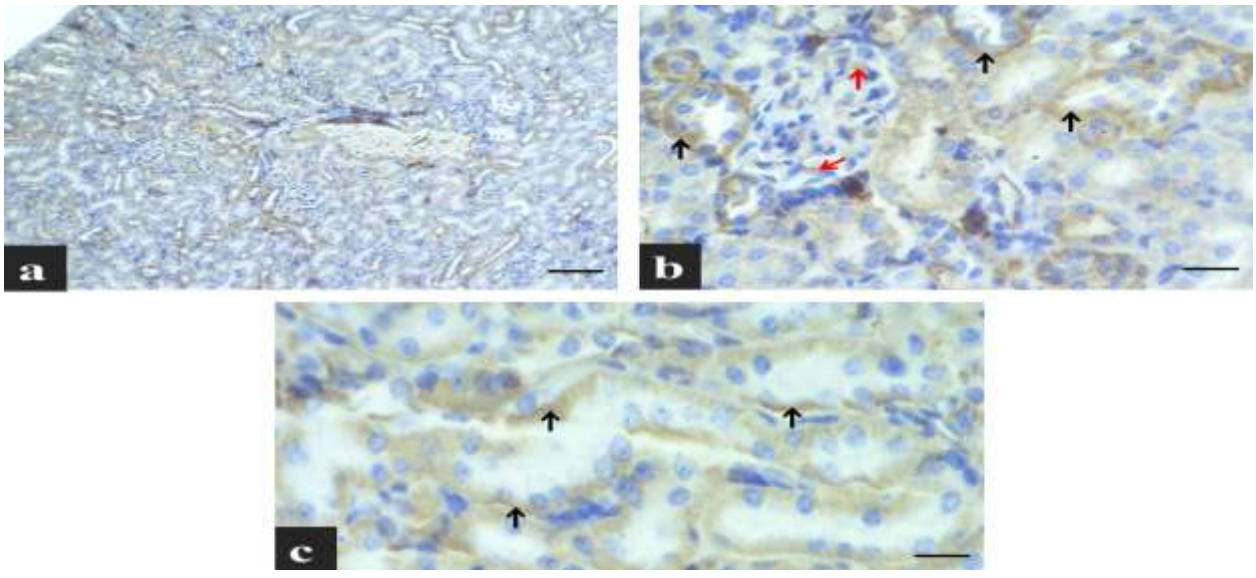

Figure 4.40:- Expression of myeloperoxidase in kidney section in mice of cisplatin + 5mg/kg of memantine treated group G4. a-c: Moderate cytoplasmic staining (score 2), in section b the black arrows showed MPO expression in proximal convoluted tubules, and the red arrows showed mesangial cell expression of myeloperoxidase, in section c the arrows showed MPO expression in Henle loops, (scale bar 100µm, scale bar 20µm).

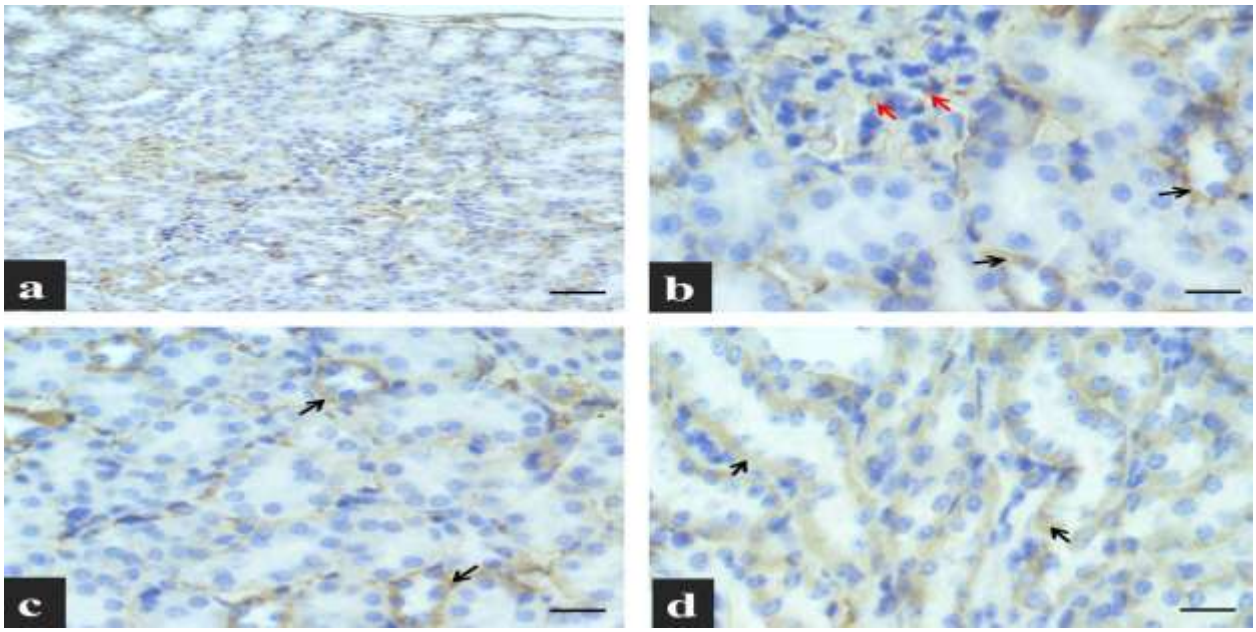

Figure 4.41: Expression of myeloperoxidase in kidney section in mice of cisplatin + 10mg/kg memantine treated group G5. a-d: Weak-diffuse cytoplasmic staining (score 1), in section b and c the black arrows showed expression in proximal convoluted tubules, and the red arrows showed mesangial cell expression of MPO, in section d the arrows showed expression in Henle loops, (scale bar 100µm, scale bar 20µm).

4.6. Immunohistochemical results of α7 nAChRs:-

Immunostaining for nicotinic acetylcholine receptors **α7 nAChRs** were independently scored by 2 readers on a scale from 0-4 where

0 = no staining,

1 = focal weak staining,

2 = focal medium staining,

3 = diffuse medium staining,

4 = diffuse strong staining (Aracava et al., 2005, Dziewczapolski et al., 2009)

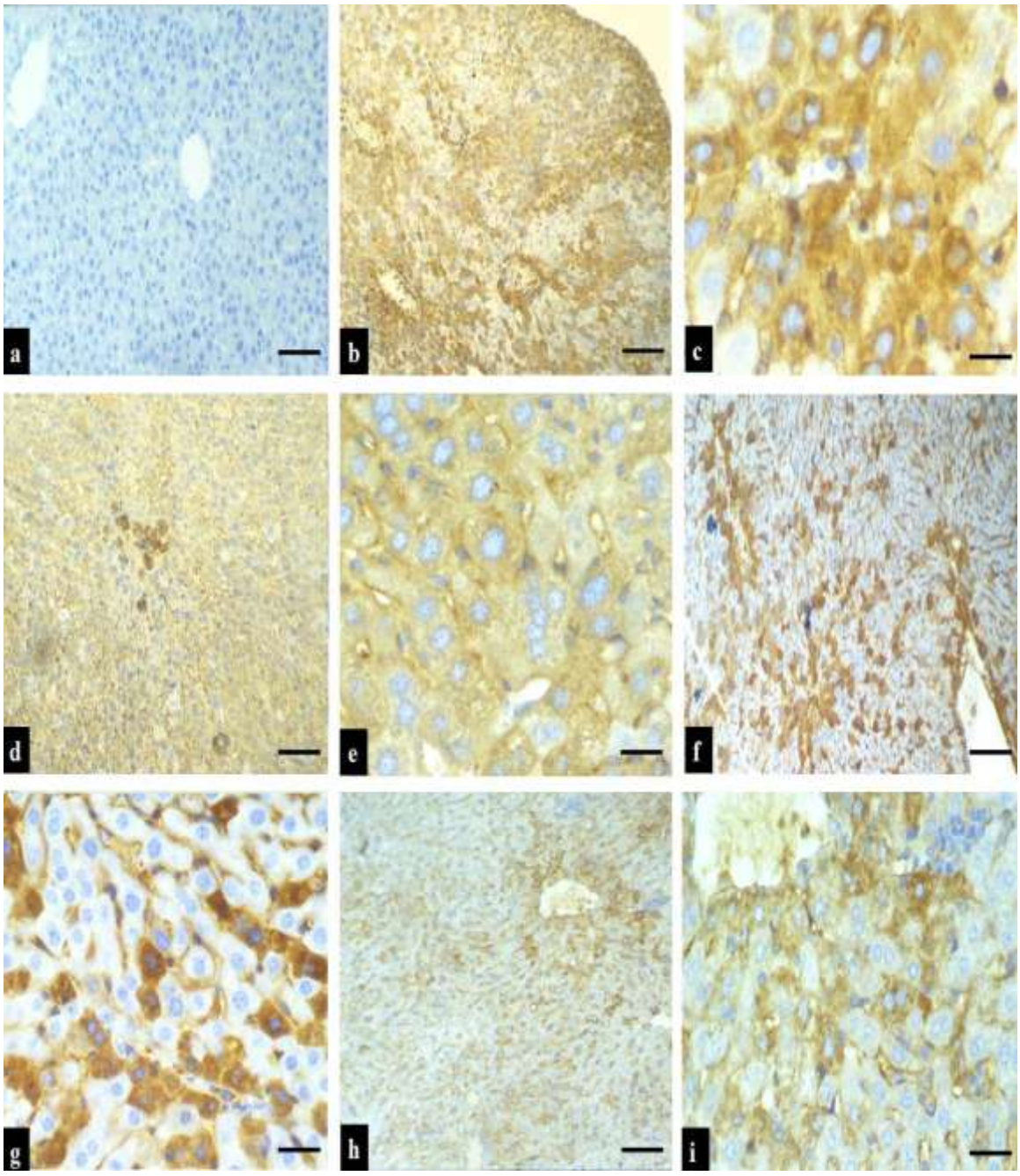

Figure 4.42: Cytoplasmic expression of α7 nAChRs in liver parenchyma in different groups; a: Negative expression (score 0) in control group G1, b and c: Diffuse strong staining (score 4) in cisplatin group G2, d and e: Diffuse medium staining (score 3) in cisplatin+ 5mg/kg of memantine treated group G4, f and g: Focal medium staining (score 2) in cisplatin+ 10mg/kg of memantine treated group G5, h and i: Focal weak staining (score 1) in memantine treated group G3, (scale bar 50 μm, scale bar 20 μm).

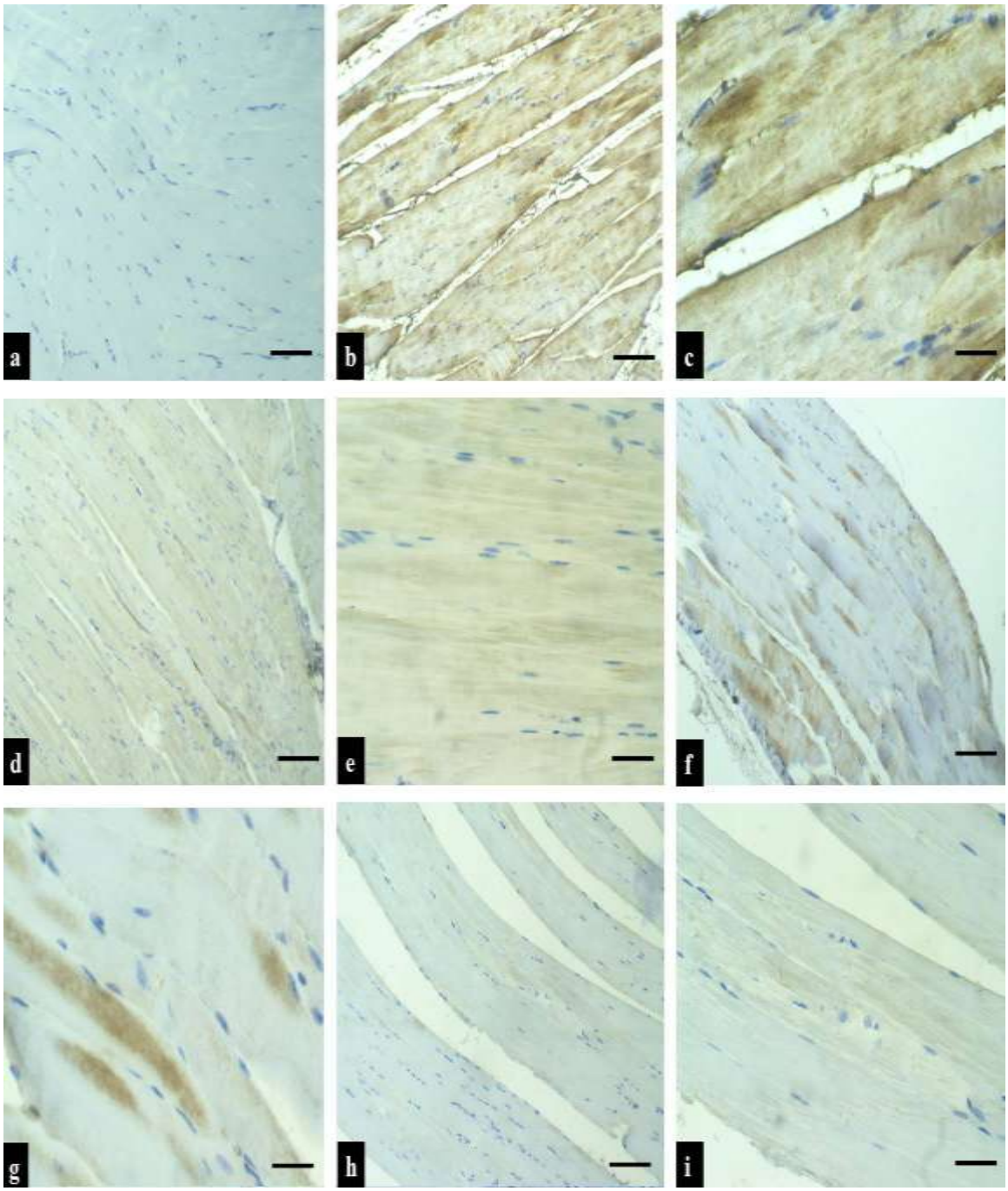

Figure 4.43: Cytoplasmic expression of α7 nAChRs in the longitudinal section of skeletal muscle in diverse groups; a: Negative expression (score 0) in control group G1, b and c: Diffuse strong staining (score 4) in cisplatin group G2, d and e: Diffuse medium staining (score 3) in cisplatin+ 5mg/kg of memantine treated group G4, f and g: Focal medium staining (score 2) in cisplatin+ 10mg/kg of memantine treated group G5, h and i: Focal weak staining (score 1) in memantine treated group G3, (scale bar 50 μm, scale bar 20 μm).

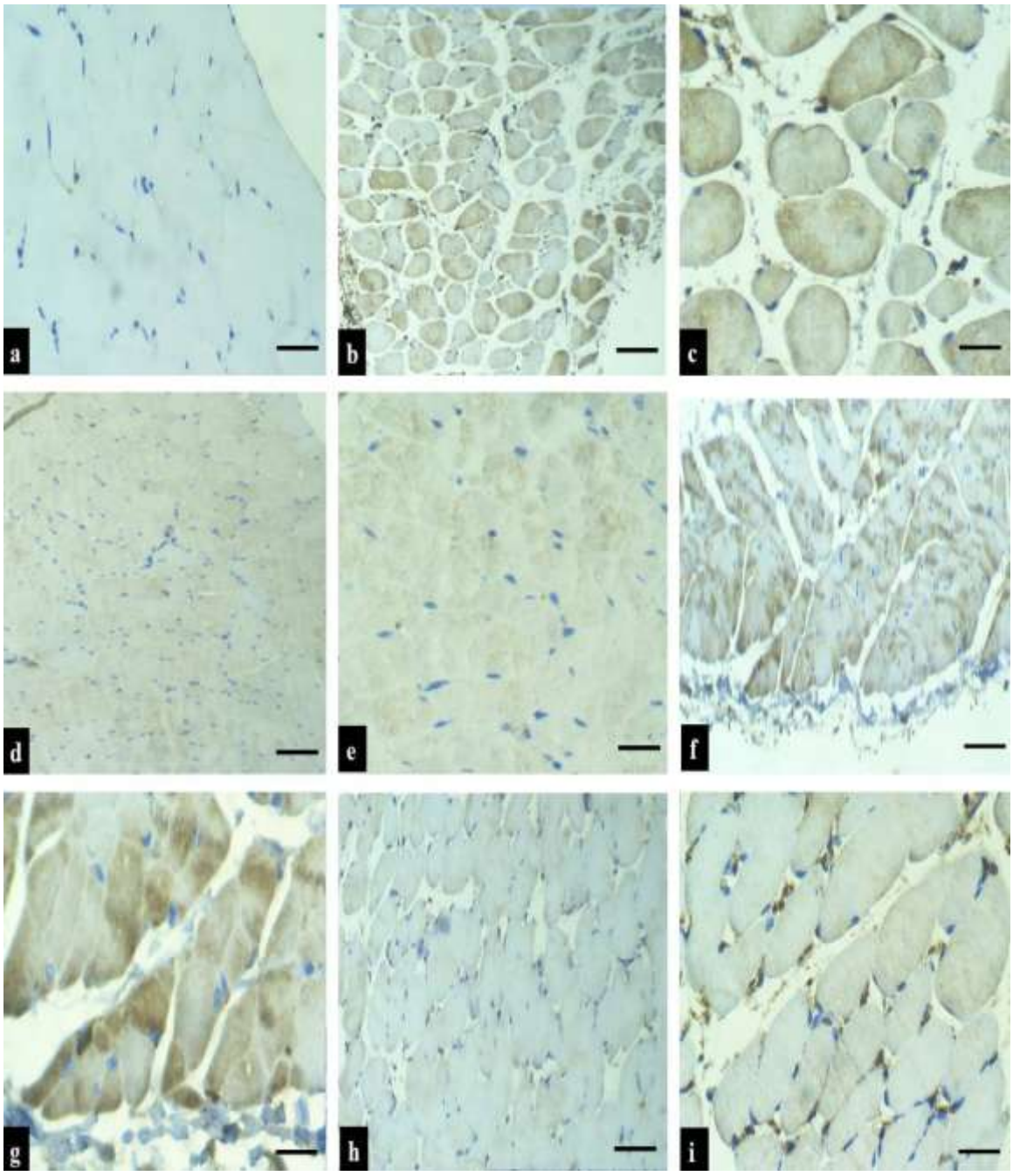

Figure 4.44: Cytoplasmic expression of α7 nAChRs in the transverse section of skeletal muscle in various groups; a: Negative expression (score 0) in control group G1, b and c: Diffuse strong staining (score 4) in cisplatin group G2, d and e: Diffuse medium staining (score 3) in cisplatin+ 5mg/kg of memantine treated group G4, f and g: Focal medium staining (score 2) in cisplatin+ 10mg/kg of memantine treated group G5, h and i: Focal weak staining (score 1) in memantine treated group G3, (scale bar 50 μm, scale bar 20 μm).

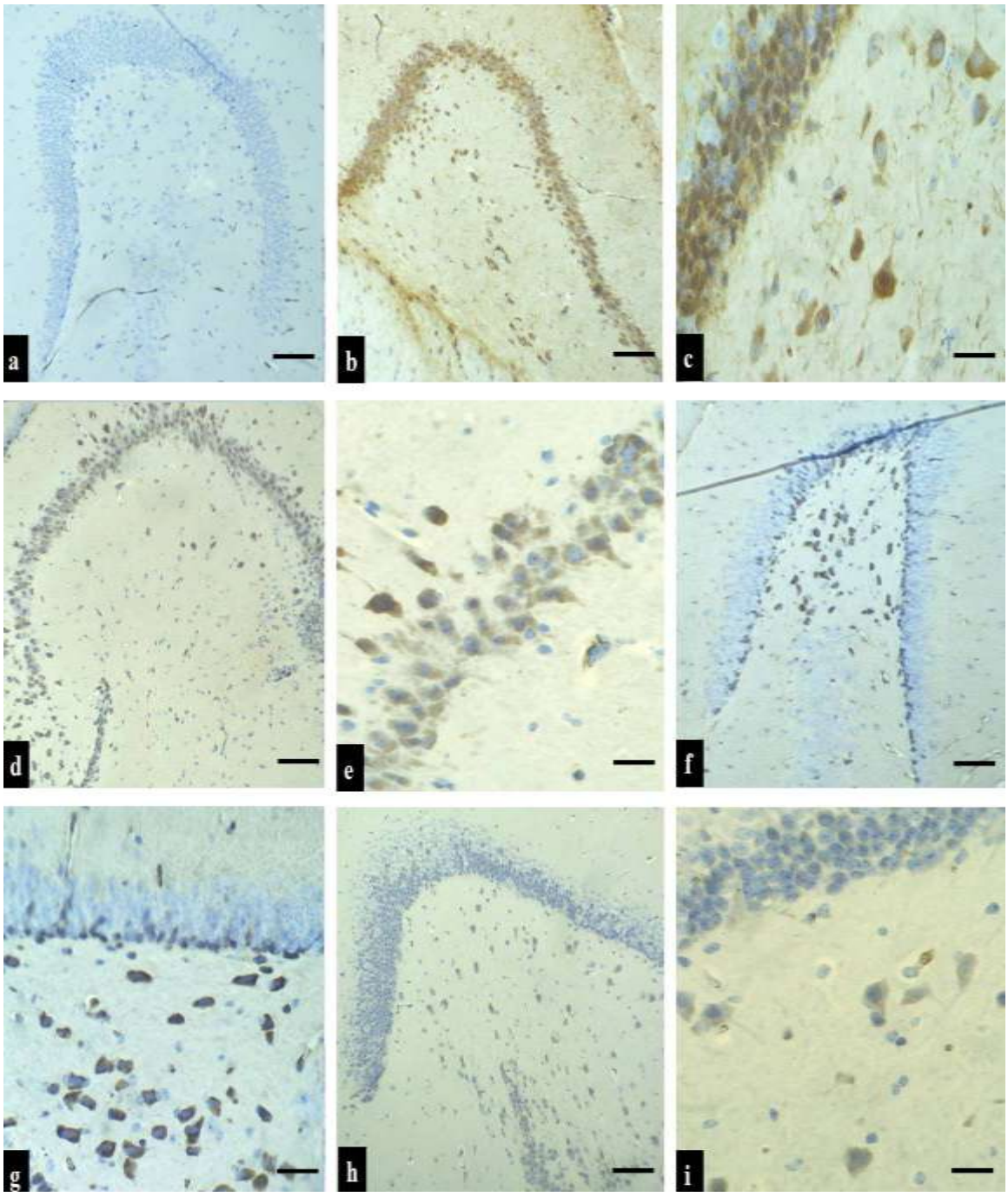

Figure 4.45: Cytoplasmic expression of α7 nAChRs in the hippocampus section in different groups; a: Negative staining (score 0) in control group G1, b and c: Diffuse strong staining (score 4) in cisplatin group G2, d and e: Diffuse medium staining (score 3) in cisplatin+ 5mg/kg of memantine treated group G3, f and g: Focal medium staining (score 2) in cisplatin+ 10mg/kg of memantine treated group G5, h and i: Focal weak staining (score 1) in memantine treated group G3, (scale bar 50 μm, scale bar 20 μm).

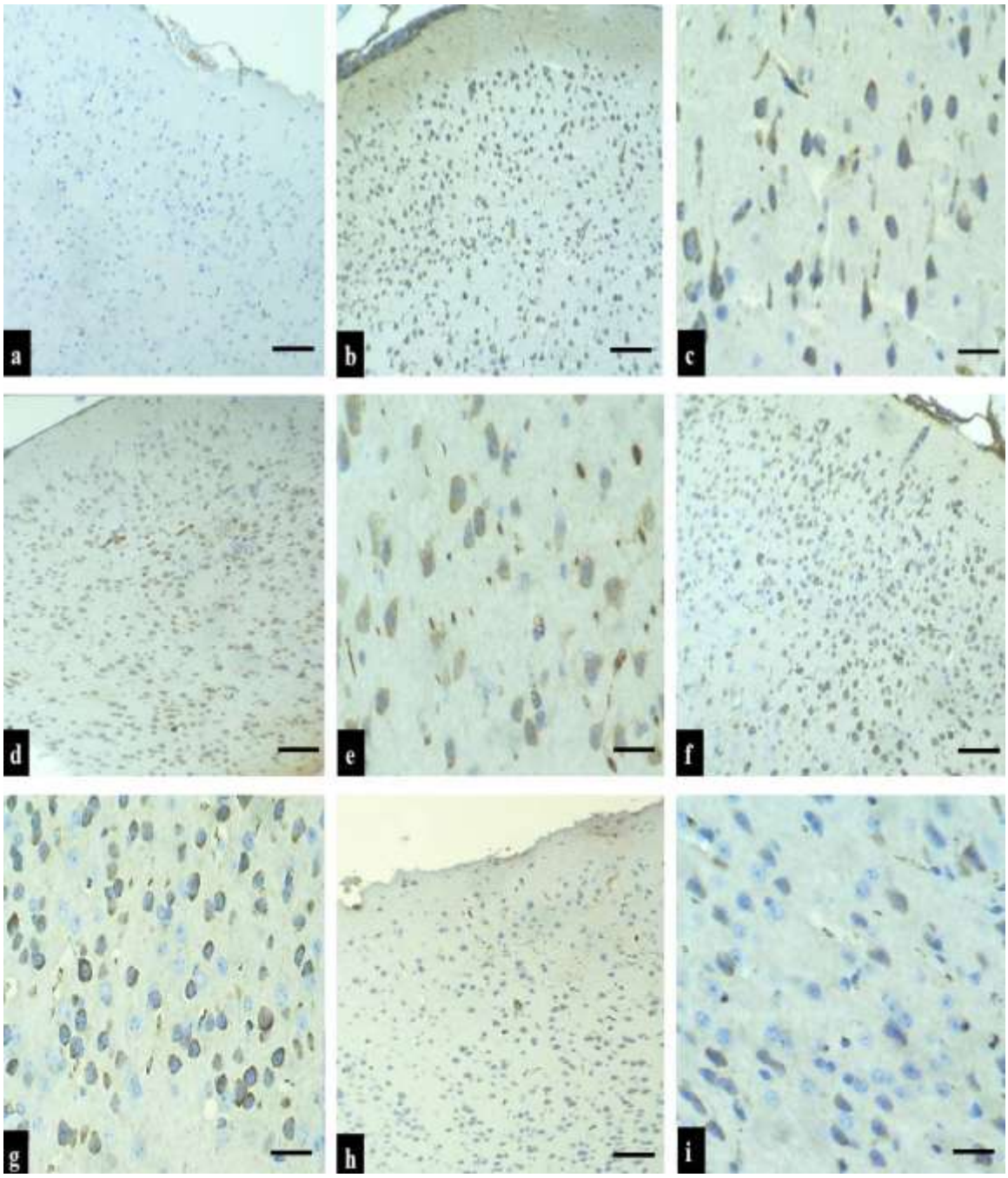

Figure 4.46: Cytoplasmic expression of α7 nAChRs in the cerebral section in different groups; a: Negative staining (score 0) in control group G1, b and c: Diffuse strong staining (score 4) in cisplatin group G2, d and e: Diffuse medium staining (score 3) in cisplatin+ 5mg/kg of memantine treated group G4, f and g: Focal medium staining (score 2) in cisplatin+ 10mg/kg of memantine treated group G5, h and i: Focal weak staining (score 1) in memantine treated group G3, (scale bar 50 μm, scale bar 20 μm).

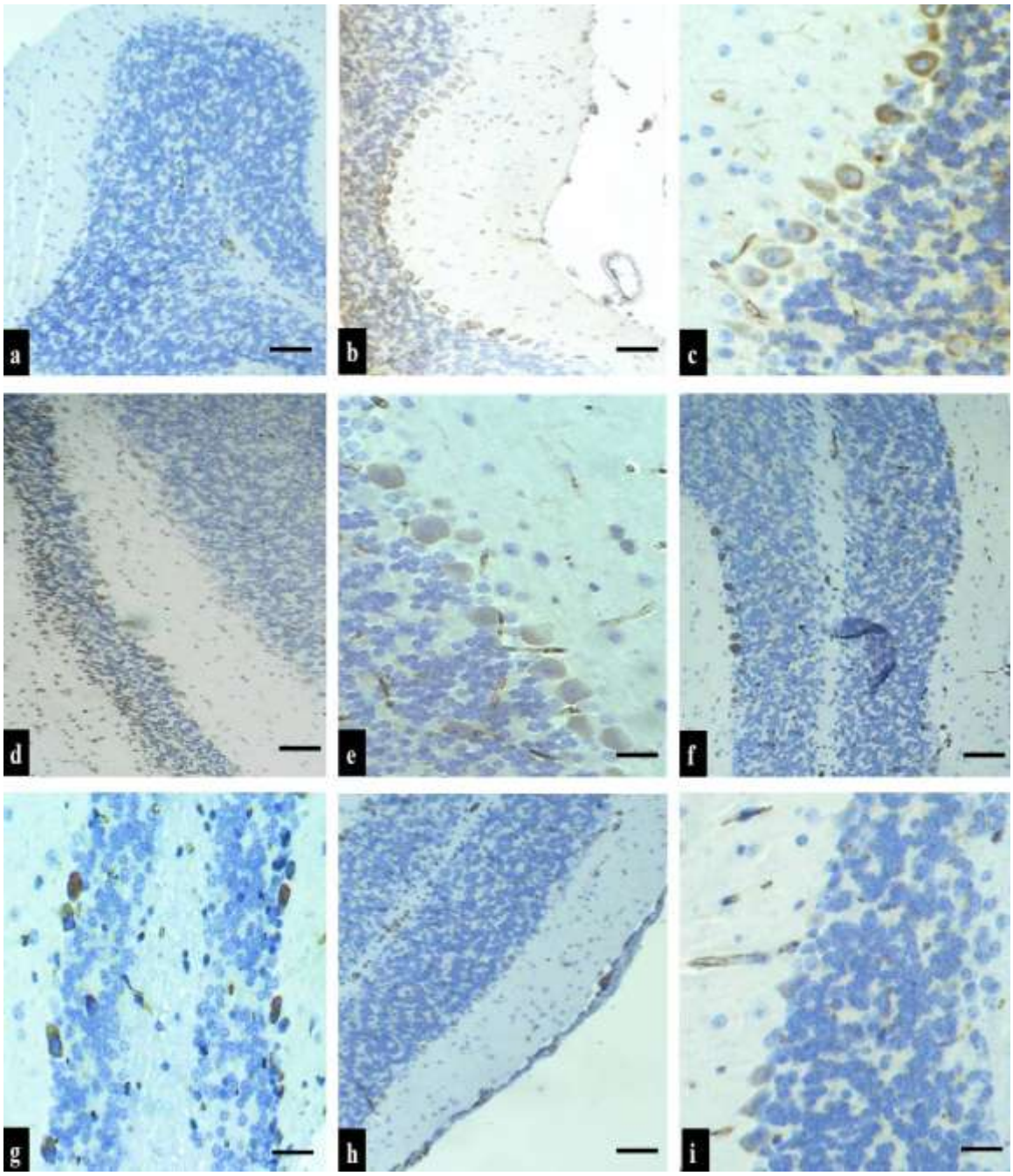

Figure 4.47: Cytoplasmic expression of α7 nAChRs in cerebellum section (particularly in purkenji cells) in different groups; a: Negative staining (score 0) in control group G1, b and c: Diffuse strong staining (score 4) in cisplatin group G2, d and e: Diffuse medium staining (score 3) in cisplatin+ 5mg/kg of memantine treated group G4, f and g: Focal medium staining (score 2) in cisplatin +10mg/kg of memantine treated groupG5, h and i: Focal weak staining (score 1) in memantine treated group G3, (scale bar 50 μm, scale bar 20 μm).

Chapter five
DISCUSSION

Antineoplastic agents are widely used for treating different kinds of cancers; but due to its highly toxic side effects; cisplatin use is limited, which may be caused by liberation of free radicals and rise of overall oxidation status of the tissues and organs, studies exist regarding the action of memantine hydrochloride to reduce oxidative stress and improve health aspect of the body. Therefore this study was conducted to examine the protective effect of memantine on nephrotoxicity, hepatotoxicity and neurobehavioral toxic adverse effects of cisplatin (Stewart and Bolt, 2012, Hill et al., 2008, Arany and Safirstein, 2003).

Memantine is a recent class of anti-Alzheimer's disease agents with a medium affinity, and uncompetitively antagonizes N-methyl-D-aspartate (NMDA) receptor. It has been proved that NMDAR activation leads to an increased enrolment of leukocytes, neutrophil, and macrophage, which leads to the induction release of glutamate, which further exaggerates the toxic status (Dimri et al., 2012, Bennett et al., 1980, Amin and Hamza, 2006, Tiwari et al., 2011, Topdag et al., 2012). When a huge amount of glutamate is released by activating it with agonists, it allows extra calcium to enter into the cells causing damage and the generation of oxidation via production of free radicals. Memantine adheres to the same glutamate receptors, blocking it, and block the entrance of too much calcium into the cells (Chipana et al., 2008, Meisner et al., 2008, Bardgett et al., 2003).

Memantine's activity as an NMDA antagonist is beneficial to improve body function on the cellular and mechanistic level and many crucial cells recruitment in

order to improve health and immunity status (Danysz et al., 2000b, Bardgett et al., 2003).

Results of the **first experiment; acute toxicity in mice** measured by up and down method showed that the acute toxic dose (LD_{50}) of cisplatin was 15.48 mg/kg which is injected by intraperitoneal route, while the acute toxic dose (LD_{50}) of memantine was 17.48 mg/kg by the oral route. They were compatible with studies carried out by (Leite et al., 2012a, Boulikas, 2004) who confirmed the acute toxic doses of both cisplatin and memantine in mice.

Results of the **second experiment**, from chosen clinical chemistry and biochemical parameters (Scr, BUN, ALT, AST, ALP, and CK) were in agreement with possible cisplatin's toxic effect on kidney and liver.

Kidneys excrete waste end product of the body such as creatinine through the glomerular filtration; any measured abnormality of this product indicates decreased excretion or impaired renal function. Therefore, creatinine regarded as estimation indicator of the kidney filtration rate. Animals from G2 and al G4 showed a significant increase on the statistical level of ($P< 0.05$**)**. The level of serum creatinine increased 2 or 3 folds especially in the G2 group, in comparison with the control group G1 or pretreatment groups with memantine G5.

Blood urea nitrogen test is indicative of altered renal function therefor, it is among the dependable indicator tests to estimate renal function (Levinson, 1978). Animals injected with cisplatin G2 showed a significant increase on the statistical level of ($P< 0.05$**)**. The level of BUN increased 2 or 3 folds especially in the G2 group, in comparison with the control group G1 or pretreatment groups with memantine G4 and G5.

ALP, AST, and ALT are dependable parameters in this study; they are hydrolytic enzyme associated with microvilli of secretory and absorptive cells. It can be taken as an indicator of the state of liver, kidney and muscle stress (Luo et al., 2008, Proksch et al., 1973, Topdag et al., 2012). Mice injected by cisplatin significantly showed an increment, while in combination treatment with either selected recommended doses of memantine (low and high dose) the values were substantially decreased.

One of the biochemical parameters which is used as a dependable test for determining any neuromuscular toxic effect is creatine kinase, observed in cardiac muscle damage and skeletal muscle disease. Any rise in the level of this enzyme means injury and stress abnormalities in the muscles, as this enzyme is rarely present in the blood. During alterations in muscle, the enzyme cleaves the muscle towards the bloodstream (Peters et al., 2015, Ibrahim et al., 2017, Levinson, 1978).

Those mice injected intraperitoneally with cisplatin G2 showed a significantly increased level of creatine kinase at the end of the treatment period. While In combination treatment with memantine, the level of creatine kinase in the serum remained normal in G5 while significantly increased in both G2 and G4

Those results obtained from above parameters in the current study are in agreement with previous studies that showed significant increase in cisplatin treated group in the levels of BUN, CK, Scr, ALP, ALT and AST(Kumar et al., 2011b, Abdelmeguid et al., 2010).

Cisplatin uptake in the kidneys is done by Organic cation transporter-2 (OCT2). Therefore, interference with cisplatin uptake via OCT2 by cationic drugs like memantine, have a protective effect through inhibition of cisplatin transport by OCT. OCT2 is most vigorously expressed in the kidney but also in the small

intestine, lung, skin, placenta, brain, choroid plexus and dorsal root ganglia (El-Arabey, 2015, Jiang et al., 2004, Shibata et al., 2007, Sprowl et al., 2013).

It was evidenced from the outcomes of the hematological parameters that the administration of cisplatin leads to increase in WBCs, and a depletion of RBCs, HB%, and PCV% which may be happened by myelosuppression or platelet aggregation. The elevation of the white blood corpuscles (WBCs) count might result from infections occurs during cisplatin treatment or a cascade of inflammatory reactions. Moreover, it was demonstrated that cisplatin induces oxidative stress injury in human thrombocytes and lymphocytes which leads to apoptosis by affecting their lifespan. Pre-treatment with memantine exhibited beneficial effects on the hematopoietic system by elevating RBCs, Hb%, and PCV % against cisplatin-induced hematotoxicity markers (Peters et al., 2015, Moore and Crom, 2006).

Blood is the most critical systems in the body. To evaluate the hazardous effect of poisons and drugs, such a relationship could be explained through the destruction of the cell of bone marrow or an increase in the fragility of RBCs. Thus, cisplatin intoxication might lead to anemia which characterized by suppressing the activity of either the hematopoietic tissues or the impaired erythropoiesis, which accelerates RBCs destruction because of the altered permeability of the RBCs membrane. The increased mechanical fragility of the RBCs memantine was found to have beneficial effects against cisplatin-induced suppression in most of the hematological parameters (Peters et al., 2015, Weinstein et al., 2000b).

Cisplatin covalently binds to the cysteine residues and forms adducts with sulfhydryl groups on hemoglobin, resulting in the loss of heme part of hemoglobin molecules, thereby reducing the Hb percentage in the blood, which cause the anemic

alteration that observed in the present investigation (Moore and Crom, 2006, Boulikas and Vougiouka, 2004).

Results from the current study are in agreement with studies done by (Cheeseman, 1993, Chen et al., 2013, Peters et al., 2015) they reported that the hematological results from the administration of cisplatin leads to increase in leukocytes, and depletion of RBCs, HB% and PCV which may be formed by myelosuppression or platelet aggregation.

Cellular targets affected by oxidative stress include DNA, phospholipids, proteins. At high concentrations, ROS can be important mediators of damage to cell structures (Chen et al., 2008a, Chen et al., 2008b). Protection of the body from oxidative stress done by enzymatic and non-enzymatic antioxidants. The current study showed a significant decline in these enzymes (GSH, GPX, CAT, GST and SOD) in mice injected with cisplatin G2 in comparison with control group, which may be obtained by impairment of GSH metabolism in a manner less GSH available to conjugate to lipid peroxidative products, by its elevation provides negative feedback on antioxidant enzymes (Topdag et al., 2012, Egashira and Takayama, 2002, Noce et al., 2015).

Studies done by (Bhandari et al., 2008, Danysz et al., 2000a, Luo et al., 2008, Topdag et al., 2012, Zhang et al., 2004d) suggested that cisplatin has an adverse effect on the function of mitochondria and encourages the synthesis of radicals and lipid peroxidation of the membrane, and DNA damage .

Synthesis of free radical and altered oxidation status of tissues has a negative impact in many kidney and liver diseases and it is complications (Ali and Al Moundhri, 2006, Leite et al., 2012b). Antioxidant enzymes protect the tissues from oxidative stress. An altered balance between synthesis of radicals, enzymatic and

non-enzymatic level with scavenging ability leading to oxidative damage of the cell components (Al-Malki and Sayed, 2014, Amin and Hamza, 2006). Kidney and livers are the most vulnerable organs to be damaged by free radicals, because of the majority of poly-unsaturated fatty acid on the lipid composition (Ali and Al Moundhri, 2006, Al-Badrany and Mohammad, 2007).

In this study, cisplatin treated group G2 showed increased levels of MDA were associated with low levels of GSH in comparison to control group G1, these results are in agreement with previous studies (Blumenthal et al., 2000, Bhandari et al., 2008).

The nephrotoxic and hepatotoxic effect of cisplatin is via the binding of cisplatin to GSH and the subsequent metabolism of the cisplatin-GSH complex (a platinum- GSH conjugation) by a γ-glutamyl transpeptidase (GGT)-dependent pathway in the proximal tubules. Elevation of ROS destroys the lipid components of the cytoplasmic membrane and cause denaturation of proteins and nucleic acids. Previous studies using rats showed an obvious decrease in these enzymatic antioxidant activities in mice treated with cisplatin (Boulikas and Vougiouka, 2003, Ali et al., 2008a, Johnsson and Wennerberg, 1999, Carvour et al., 2008).

Myeloperoxidase (MPO) regarded as a heme molecule -containing peroxidase highly abundant in neutrophils. MPO is able to produce hypochlorous acid from the hydrogen peroxide by the presence of chloride ions which is a unique action of this enzyme, when these neutrophils are stimulated by different stimulants, MPO level will increase as other cellular tissue-damaging agents, the MPO-hydrogen peroxide-chloride system initiate lipid adducts which leads to kidney malfunction (Kumar, 2011, Dabas et al., 2012). The sticking of neutrophils to the glomerular membrane, activation of platelets, and subsequent proliferative responses produce

inflammatory and proliferative glomerulonephritis and destruction by oxidants at sites of attachment this fact prove the direct engagement of MPO (Walia et al., 2011, Boulikas and Vougiouka, 2003, Zhang et al., 2004a).

Immunohistochemistry result of the present study confirmed the presence of higher score of MPO in cisplatin-treated group G2 when comparing it with that of the control group G1 and another treated groups of both combination treatments groups of memantine G4 and G5.

The current study demonstrated that cisplatin increases the AChE expression level in cisplatin-treated mice G2 in comparison with mice treated with memantine alone G3 and in combination treatment G4 and G5. The increment level of ROS and altered calcium balance might result in an increase in AChE activity. A study on rat has reported that the ameliorative effect of memantine against cognitive dysfunction, such effect is mediated through reduction of hippocampal AChE level (Zhu et al., 2015, Song et al., 2008b).

Induction of free radical formation by cisplatin may upset the oxidation and antioxidation balance within the brain, which the most possible reasons of increase in the AChE activity, who suggested that cisplatin, initiate synaptic dysfunction, which means adduction of presynaptic protein thiol groups and subsequent alteration in neurotransmitter release, neurological alterations are predisposed by deficit neurotransmission at the neuronal synapses (Rogers et al., 2003, Danysz et al., 2000a, Sen, 2000).

The authors explained the increase in AChE to the compensatory mechanism of a brain to long-term administration of cisplatin and sustained glutamate activation of N-methyl-D-aspartate receptors which lead to the up-regulation of AChE activity

leading to neuronal injury and cognitive deficits. Cisplatin has more affinity for α7 nAChRs receptor than its physiological agonist like Ach (Volbracht et al., 2006, Dong et al., 2017, Danysz et al., 2000a, Takizawa et al., 1990).

Cisplatin resulted in a significant rise in dopamine (DA) and acetyl cholinesterase (AChE). Interestingly this compound declined serotonin (5HT) level. Studies suggest that altered 5-HT release play the major role in the cognitive abnormality in patients treated with the platinum antineoplastic agent, these results indicate that neurotransmitter release impairment play major role mechanism of cognitive dysfunction in patients treated with chemotherapeutic agents. α7 nAChRs and the 5-HT3 exhibited cross-pharmacological relation, e.g. increase concentrations of the α7 nAChRs agonist nicotine inhibit 5-HT3 receptor-mediated responses (Aracava et al., 2005, Arany and Safirstein, 2003, Thomson, 2019, Bardgett et al., 2003, Wang et al., 2015).

Cisplatin caused a decrease in serum calcium, potassium, and sodium percentage. While memantine caused an insignificant change in the serum calcium, potassium and sodium levels these results are in agreement with previous studies (Cai et al., 2004, Reynolds et al., 2004, Frasco et al., 2005, Zhu et al., 2015).

Immunohistochemical staining of nicotinic acetylcholine receptor (nAChRs) showed a protective role of memantine G3; in which G2 yielded the highest intensity score while G3 and G5 yielded the lower scores in comparison with G1. In a study done by (Dziewczapolski et al., 2009, Gitto et al., 2002) reported that deletion of the α7 nAChRs gene improved the cognitive deficits displayed in the water maze by a mouse model. The authors, therefore, suggested that the antagonism of α7 nAChRs function could be pro-cognitive, they point out that α7 nAChRs agonists and

antagonists share certain effects; that the rapid down-regulation of α7 nAChRs following its activation quite difficult to distinguish agonist from antagonist action at α7 nAChRs.

Cisplatin induced α7 nAChRs activation were identified to be associated with proteins conformation changes characterized by the opening of transmembrane calcium channels. Therefore, the functional states of α7 nAChRs changed rapidly and cisplatin treatment cause α7 nAChRs up-regulation, which serve as a biochemical marker of the synaptic neurotransmission impairments, learning and memory dysregulation in animals In neuronal cells up-regulation of the α7 nAChRs promotes an increase in intracellular Ca2+ through voltage-activated channels and glutamate (Dziewczapolski et al., 2009, Vladimirov and Proskurnina, 2009, Gopal et al., 2012).

All (mitogen-activated protein kinase) MAPK pathways appear to have been activated in vulnerable neurons to neurotoxicity, suggesting their involvement in the pathophysiology and pathogenesis of cisplatin treatment in a manner dependent on calcium and α7 nAChRs, the presence of α7 nAChRs sensitizes the cells to toxicity induced by prolonged exposure to cisplatin, as measured by MAPK Pathways induced Oxidative Stress, increased level of ROS production, altered nuclear integrity, and eventually cell death. Cisplatin treatment causes α7 nAChRs up-regulation concomitantly with dysregulation of extracellular signal-regulated kinase (ERK2) MAPK. Considering that ERK MAPK activity is necessary for rodent spatial learning, α7 nAChRs up-regulations in hippocampus may serve as a biochemical marker for the synaptic plasticity impairments and learning and memory deficits in animals, cisplatin-induced ERK activation is a key regulator of the p53 response to

DNA damage caused by cisplatin. Increased ERK activation was followed by increased levels of reactive oxygen species (ROS). (Jiang et al., 2007, Zhang et al., 2004c, Aracava et al., 2005, Dziewczapolski et al., 2009, Gitto et al., 2002).

Cisplatin produced several neurological deficits, including hind-limb foot splay, decreased grip strength, ataxia, and skeletal muscle fatigue, which may be related to the degeneration of the brain monoaminergic system. cisplatin treatment-induced alterations in the level of brain monoamines and photomicrograph changes ranging from focal gliosis in the cerebral cortex and the cerebrum, hemorrhage in the meninges, and vacuolization in the cerebral cortex, cerebrum, cerebellum, and medulla oblongata in comparison with control group which show normal histological feature while memantine treated group either alone G3 or in combination treatment groups G4 and G5 which show little histological changes (Peters et al., 2015, El-Arabey, 2015, Chipana et al., 2008). Slides from memantine treated groups, either alone as in G3 or in combination with cisplatin as in G4 and G5, showed no morphological changes and reflected the impact of memantine in preventing the appearance of hepatic abnormal changes (Ali et al., 2008a, Johnsson and Wennerberg, 1999, Chipana et al., 2008).

The structural histopathological differences and rate of renal parenchymal accumulation between cisplatin and memantine may contribute to major toxic adverse effects of cisplatin according to the dose used (Punithavathi et al., 2011, Ali et al., 2008a, Balakrishna et al., 2011, Flores et al., 2011).

In the **third experiment**, weights of animals in cisplatin-treated groups G2 showed a significant loss of weights in treatment period in comparison with control group G1, while groups G4 and G5 showed complete protection from the adverse effect of cisplatin, the negative effect of cisplatin on weight gain caused by cisplatin

due to imbalance of energy metabolism, metabolic transformation and decrease in food consumption because of adverse toxic effects of cisplatin. Our results are in agreement with previous studies (Yao et al., 2007, Kounis et al., 2016).

Brain is a complicated and delicate organ which is quickly affected by chemotherapeutic agents in treating various cancers. Neurotoxicants are reported to be negatively affecting the hippocampus by stimulating the neuroinflammatory response, which in consequence causes behavioral alteration by induction the formation of nitrite oxide synthase (iNOS) level in the hippocampus that exacerbates the nitrosative stress, which later elicits the formation of nitrite oxide (NO) which is an unstable product. These nitrogen species exaggerate the process of apoptosis and neuronal cell death (Gitto et al., 2002, Zhang et al., 2004b, Sen, 2000).

Cisplatin exposure in the rat at dose 5–10 mg/kg produces several neurological alterations, including ataxia, foot splay, decreased limb grip strength, and skeletal muscle fatigue (Bardgett et al., 2003, Huang et al., 2001a, Alias et al., 2011, Huang et al., 2001b).

Neuronal degeneration and inflammation are caused by up-regulating and stimulation of NMDAR; it requires two agonists, glutamate and glycine, to activate it. When NMDAR activation occurs it induces tissue damage, through up-regulation of pro-inflammatory cytokines, such as IL-1β, TNFα, inflammatory adhesion molecules, and recruitment of leukocytes into tissues (Sharma and Kumar, 2011, Boulikas and Vougiouka, 2003, Topdag et al., 2012, Peters et al., 2015)

In the present study, we found marked motor in-coordination in the cisplatin-treated mice which is evident by the results of swimming, hole-head test, negative geotaxis, and open field test. In G2 there was a significant decrease in the number of

squares crossed, head dipping, and swimming score by the mice while in G3, G4 and G5 there is insignificant changes in comparison to the control group G1. In terms of negative geotaxis, G2 exhibited a significant increment in the time that is necessary by the mice for correcting the body position and this was positively proportional to the treatment period. In G4, the values started to increase after four weeks of treatment. Studies have proofed that cisplatin treatment lead to motor imbalance and incoordination by negatively altered the growth of Purkinje cells and morphology of granule cells (Dziewczapolski et al., 2009, Dong et al., 2017)

Several pharmacological studied explained that NF-κB plays a critical role in the activation of the neuroinflammatory pathway which causes the formation of iNOS gene expression level and proinflammatory cytokines level. The raised level of proinflammatory cytokines such as TNF-α and IL-1β were observed after weekly treatment of cisplatin in rat for seven weeks (Dong et al., 2017, Gopal et al., 2012).

Experimental studies suggested that the neurotoxic action of cisplatin-mediated via abnormalities in the structural feature of BBB which permit drug passage of drugs through it (Gelderblom et al., 2002, Rogawski and Wenk, 2003, Ilbey et al., 2009).

Treatment with memantine significantly improved the neurological deficits; this current result was in accordance with (Topdag et al., 2012, Peters et al., 2015) who reported that memantine significantly alleviated peripheral neuropathy that cause by aminoglycoside in rats, which is proved by increase body weight, improved motor coordination, increased grip strength and improved nerve conduction velocity.

Memantine at the recommended dose of (15 mg/kg/day for 28 days) reduces the amount of activated microglia which is an expression of brain inflammation also

reduced neurological deficits, diminish the brain edema and BBB passage rate (Peters et al., 2015, Belzung and Griebel, 2001).

Memantine dose of 5 mg/kg offered partial protection in renal, hepatic, neurobehavioral and hemotoxic side effects of cisplatin, while treatment with therapeutic dose of memantine 10 mg/kg obviously offered complete protection.

Chapter six

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. This study explored the potential role of memantine as a protective agent against the main side effects of cisplatin which can be beneficial for patients suffering from cancer. Memantine at a higher therapeutic dose of 10mg/kg is more effective than a lower therapeutic dose of 5 mg/kg at reserving intracellular levels of biological pathways, supportively enhancing all the main hematological, kidney and liver parameters (ALP, AST, Scr, BUN, CK and ALT) and improving the enzymatic and non-enzymatic antioxidants levels (SOD, GSH, MDA, GPx, CAT and GST) in tissue homogenate. These results are confirmed by histopathological and immunohistochemical research (MPO and α7 nAChRs). This study provides a protective strategy in the management of nephrotoxicity, hepatotoxicity and hematotoxicity induced by cisplatin.

2. This study explored the potential role of memantine as a protective agent against neurotoxicity, the main side effect of cisplatin. This can be beneficial for patients suffering from neuropathy and will also help to further uncover the critical areas of memantine hydrochloride. It can be inferred from the results of the current study that the neuroprotective effect shown by memantine in cisplatin-induced neurobehavioral toxicity can preserve intracellular levels of biological pathways and supportively enhance all the associated parameters regarding performing the main neurobehavioral parameters which are confirmed by the results of the histopathological and immunohistochemical study.

6.2 Recommendation

These results strongly suggest that memantine could be considered as a potentially useful candidate to be taken as a protective agent during treatment with cisplatin drug. However, further studies are needed in order to explore the exact cellular mechanisms underlying the cytoprotective effect of memantine and it impacts on the efficacy of cisplatin as a platinum chemotherapeutic agent in the treatment of cancer.

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Appendix (1):

Table of K value according to the Up and Down acute toxicity results.

$LD_{50} = X_f + K_d$

Second part of serial	**+ K represents serial results as follows**				
	O	**OO**	**OOO**	**OOOO**	
XOOO	-0.157	-0.154	-0.154	-0.154	**OXXX**
XOOX	-0.878	-0.861	-0.860	-0.860	**OXXO**
XOXO	0.701	0.737	+0.741	0.741	**OXOX**
XOXX	0.084	0.169	0.181	0.186	**OXOO**
XXOO	0.305	0.372	0.380	0.381	**OOXX**
XXOX	-0.305	-0.169	-0.144	-0.142	**OOXO**
XXXO	1.288	1.500	1.544	1.549	**OOOX**
XXXX	0.555	0.896	0.985	1.007	**OOOO**
	X	**XX**	**XXX**	**XXXX**	**Second part of serial**
	- K represents serial results as follows				

BIODATA OF THE STUDENT

Nadia Abdulkareem Salih is an assistant professor at College of Veterinary Medicine, University of Sulaimani, Iraq. Nadia born and raised in Kirkuk city in 1979, later in secondary school in 1996 she travelled in order to stay and live in Sulaimani city, located in the Northern side of Iraq. She got a Bachelor degree of Veterinary Science in 2003 from University of Sulaimani, and then she started her career as demonstrator at 2004-2006. She held a master's degree in pharmacology and toxicology in 2008. Her Ph.D. dissertation research focuses on the novelty ameliorating effects of memantine hydrochloride on cisplatin-induced toxicity with special reference to the neuro-, nephro- and hepato-toxicity in mice. During her Ph.D. study, participated in several conferences and workshops; also she published 14 researches during her scientific life that she used them for scientific upgrading until she became assistant professor on 2015. She published three related researches from this dissertation in Thomson Router journals with good impact factor and she obtained score 6 of English language (IELTS) during her PhD study.

LIST OF PUBLICATIONS

1. Effect of Memantine Hydrochloride on Cisplatin-induced Toxicity with Special Reference to Renal Alterations in Mice, Nadia Abdulkareem Salih and Banan Khalid AL-Baggou, International Journal of Pharmacology, ISSN 1811-7775, Int. J. Pharmacol., 15 (2): 189-199, 2019.
2. The Effect of Memantine Hydrochloride on Cisplatin-induced Toxicity with Reference to Hepatic and Haematological Alterations in Mice, Nadia A. SALIH & Banan K. AL-BAGGOU, Latin American Journal of Pharmacy Lat. Am. J. Pharm. 38 (1): 141-9 (2019).
3. Effect of memantine hydrochloride on cisplatin-induced neurobehavioral toxicity, Acta Neurologica Belgica, Nadia A. SALIH & Banan K. AL-BAGGOU , ANEB-D-18-00526 (2019) 2240-2993 (Online), acceptance letter.

الخلاصة

تعد ضادات الاورام الخبيثة من المركبات المستخدمة في علاج انواع مختلفة من السرطانات.

السسبلاتين عقار واسع الاستخدام و بسبب سميته العالية تم تحديد استخدامه كنتيجة للاذى الحاصل من اطلاق الجذور الحرة ، ومن هنا اهتمت هذه الدراسة بدور الميمانتين المختزل للجهد التاكسدي.

صممت الدراسة لإيجاد الدور الوقائي المحتمل للميمانتين المعطى عن طريق الفم للفئران المعالجة به بجرعتين مختلفتين هما 5 و10 ملغم / كغم كمحاولة للتضادد مع التاثيرات الجانبية السمية للسسبلاتين المحقون في التجويف الخلبي بجرعة 4 ملغم / كغم مع التركيز بشكل خاص على التغيرات الحاصلة في الكلية والكبد فضلا عن التغيرات السلوكية العصبية.

شملت هذه الدراسة 3 تجارب رئيسية:

في التجربة الاولى تم قياس الجرعة السامة الحادة لكلا العقارين السسبلاتين والميمانتين كل على حدة بطريقة الصعود والنزول .

في التجربة الثانية درست التأثيرات الضارة الرئيسية للسسبلاتين والتي شملت السمية الكلوية، السمية الكبدية والسمية الدموية من خلال قياس المؤشرات الخاصة بالجهد التاكسدي , كما تم اجراء الفحوصات النسيجية المرضية في مختلف انسجة الجسم و دراسة التصبيغ المناعي الكيمونسيجي للاجسام المضادة لكل من البيروكسيديز النخاعي ومستقبلات الاستيل كولين النيكوتينية.

اظهرت الفئران المحقونة بالسسبلاتين عبر التجويف الخلبي زيادة معنوية في مستوى كل من خميرة الالنين امينوترانسفيريز وخميرة الاسبارتيت امينو ترانسفيريز ونيتروجين يوريا الدم وخميرة الفوسفاتيز القاعدية كذلك زيادة في مستوى الكرياتنين المصلي ومستوى المالوندايالديهايد والكرياتين كاينيز وخميرة الاستيل كولين استراز في المصل ، و زيادة في درجة تنظيم مستوى البيروكسيديز النخاعي ومستقبلات الاستيل كولين النيكوتينية مع احداث تذبذب كبير في مستويات كل من الكلوتاثيون، الكلوتاثيون ترانسفيريز ،السوبر اوكسيدميوتيز ،الكاتاليز والكلوتاثيون بيروكسديز، ومستوى الدوبامين و السيروتونين و الصوديوم والكالسيوم والبوتاسيوم فضلا عن الفحوصات النسيجية المرضية لكل نسيج وفضلا عن ذلك، فقد أظهرت الفحوصات المرضية النسيجية أذىً ملحوظاً في أنسجة الكلية

والدماغ والعضلات الهيكلية والكبد والرئتين ونسيج الخصى التي عكست مؤشرات على الاذى الملحوظ فيها.

اظهرت الفحوصات الدموية في الفئران المعاملة بالسسبلاتين زيادة معنوية في عدد خلايا الدم البيضاء مع هبوط وتراجع في كل من عدد كريات الدم الحمر وحجم الخلايا المرصوصة ومستوى خضاب الدم حيث تعد هذه القياسات المعيار الرئيسي للسمية الدموية.

اجريت التجربة الثالثة لغرض الكشف عن دور الميمانتين الضاد للسمية السلوكية العصبية المحدثة بالسسبلاتين ، حيث سجلت القراءات السلوكية العصبية اسبوعيا ودرست على مدى 30 يوما باستخدام مجموعة من الفحوصات التي شملت الاختبارات السلوكية العصبية مثل نشاط الميدان المفتوح واختبار الانتحاء الارضي السالب واختبار اللوح المثقوب واختبار السباحة. اظهرت الفئران المعاملة بالسسبلاتين انخفاضا معنويا في القدرة على القيام بالمهام السلوكية العصبية مع انخفاض في اكتساب الوزن مقارنة بمجموعة الفئران المعاملة بالسسبلاتين والميمانتين معا والتي اظهرت تحسنا معنويا في القدرة ونمط اكتساب وزن الجسم بشكل طبيعي.

في الختام دلت نتائج هذه الدراسة على الدور الفاعل للميمانتين كمضاد للتاثيرات الضارة للسسبلاتين في ما يتعلق كل بالسمية الكلوية و السمية الكبدية وعلى مستوى السمية السلوك العصبي والتي وفرت وقاية جزئية بجرعة الميمانتين 5 ملغم / كغم من وزن الجسم ووقاية كلية له بجرعة 10 ملغم / كغم من وزن الجسم.

تأثيرميمانتين هايدروكلورايد في تقليل التاثيرالسلبي للسيسبلاتين من حيث السمية الكلوية والكبدية والتسمم العصبي في الفئران

اطروحة مقدمة إلى مجلس كلية الطب البيطري في جامعة السليمانية

كجزء من متطلبات نيل شهادة الدكتوراة في الطب البيطري/

Veterinary Pharmacology and Toxicology

من قبل

نادية عبد الكريم صالح

بـإشـــراف

الاستاذ المساعد الدكتورة بنان خالد البكوع

٢٠١٩ م

پوختە

ئەم توێژینەوەیە پێکهاتووە لە سێ تاقی کردنەوە بە مەبەبەستی دەرخستنی کاریگەری میمانتین هایدروکلوراید لە کەم کردنەوەی یان دژایەتی کردنی کاری ژەهراوی سیسبلاتین وەکو: ژەهراوی بوونی گورچیلە، ژەهراوی بوونی جگەر و میشك.

یەکەم تاقی کردنەوە تەرخان کرا بوو بۆ لێکولینەوە و پێوانە کردنی ئاستی ژەهراوی بوون بەم دەرمانانە بە ڕێگای (up and down method) وە دەرخستنی ئاستی (LD_{50}) .

دووەم تاقی کردنەوە بریتیە لە لێکۆڵینەوەی کاریگەریی دوو بڕی پێویست 5 ملغم/کغ و 10 ملغم/کغ لە دەرمانی میمانتین بەتەنها و لەگەڵ سیسبلاتین 4ملغم/کغ لە سەر لایەنی بایوکیمیاوی و ئەنزیمی وامیونوهیستوکیمستری و هستەوباسولوجی لەمشك.

ئەم تاقیکردنەوەیە یەك مانگ دەخایەنێت لە کوتایدا ئەم پارامیتەرانە بیوانەکراون:

Alanine aminotransferase, Aspartate aminotransferase, Blood urea nitrogen, Alkaline phosphatase, Serum creatinine; Malondialdehyde, Creatine kinase, Serum Acetylcholineesterase, Myeloperoxidase and Nicotinic acetylcholine receptors

لە ئەنجامدا دەرکەوت کە ئەم پارامیتەرانەی سەرەوە زیادی کردووە بە ڕێژەی بەرچاو لەو گروپانەی چارەسەرکراون تەنها بە سیسبلاتین بە بەراورد لەگەڵ گروپی کونترۆڵ بەڵام ئەو گروپانەی کە چارەسەر کراون بەمیمانتین بە هەردوو بری بیوسیت لەگەڵ سیسبلاتین کەمتر گۆڕان بە سەر ئەو پارامیتەرانە داهاتووە.

وە دەرکەوت کە ئاستی Glutathione, Glutathione transferase, Superoxide dismutase, Catalase, Glutathione peroxidase, Dopamine, Serotonin, Sodium, Calcium, and Potassium contents. لە خوێن کەم دەبیتەوە لە گشت گروپەکان بە بەکارهێنانی دەرمانی سیسبلاتین بە بەراورد لەگەڵ گروپی کونترۆڵ. ئەم ئەنجامانە ئەگەریتەوە بۆ جیاوازی لە نیوان شیوەی کیمیاوی سیسبلاتین و میمانتین و شیوەی جووڵەی ئەم دەرمانانە لەناو لەشدا. چارە سەرکردن بە میمانتین وە وای کرد کە دەرمانی سیسبلاتین کەم کۆ ببیتەوە لە گورچیلە و لە دەر ئە نجام بووە هۆی کەم کردنەوەی ژەهراوی بوون.

سێ یەم تاقی کردنەوە ئەنجام درا لە مشکدا بە بەکارهێنانی دوو بڕێ بیوست لە میمانتین بەهۆی دەمەوە و ئە نجام دانی تاقیکردنەوەی (Neurobehavioral test) وەك

Open field activity, Negative geotaxis, Hole-board test, and Swimming test.

لە ئەنجامدا دەرکەوت کە ئەم پارامیتەرانەی سەرەوە بار گۆڕان ی تەواو و بە ڕێژەی بەرچاو لەو گروپانەی نیشاندەدا کە چارەسەرکراون بە سیسبلاتین بە بەراورد لەگەڵ گروپی کونترۆڵ بەڵام ئەو گروپەی کە چارەسەر کراون بەمیانتین بە هەردوو بری بیوسیت بە تاییبەت بڕێ 10 ملغم/کغ کەمتر گۆڕانی بە سەر ئەو پارامیتەرانە داهاتووە.

هەر وەك دەرە نجامێك بۆ گشت تاقی کردنەوەکانی ئەم تۆێژینەوەیە دەرکەوت کە میانتین بە بڕێ 10 ملغم/کغ دەبیتە هۆی بارستنی زیاتر لە پارامیتەرەکان ،وە هەروەها دەرکەوت کە توانای باشی هەیە لە دژایەتی کردنی کاری گەری ژەهراوی سیسبلاتین و کەم کردنەوەیان.

کاریگەریی میمانتین هایدروکلوراید لە کە م کردنەوە ی ژەهراوی بوونی گورچیلە و جگەر وکۆئەندامی دەمار دەرئە نجامی بەکارهینانی سیسبلاتین لە مشکدا

ئەم نامەیە پێشکەشە بە کۆلیجی پزیشکیی ڤێترنەری زانکۆی سلێمانی وەک بەشێک لە پێداویستیەکانی بەدست هێنانی بڕوانامەی دکتۆرا لە بواری پزیشکیی ڤێتیرنەری/

Veterinary Pharmacology and Toxicology

لە لایەن

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