Comparative Analysis of Antioxidant Activity of *Persea americana* and *Carica papaya* against Cadmium Induced Neurotoxicity, Hepatotoxicity and Nephrotoxicity in Rats

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MS Industrial Biotechnology

Supervised By

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Islamabad

2019

DECLARATION

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(Supervisor) Dr. Tahir Ahmad

Industrial Biotechnology

ASAB, NUST.

I’d like to dedicate this thesis to the two strongest pillars of my life;

My Beloved Parents & My Best Friend Maria Sana

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LIST OF ABBREVIATIONS

AChE----- Acetylcholine esterase

ALP------- Alkaline amino transferase

ALT------- Alanine aminotransferase

CAT------- Catalases

$CdCl_2$------ Cadmium chloride

Cd---------- Cadmium

EU---------- European Union

GPx-------- Glutathione peroxidase

I**ARC**------- International Agency for Research on Cancer

LPO-------- Lipid peroxidation

MAPK----- Mitogen-activated protein kinases

MDA------- Malondialdehyde

MT--------- Metallothionein

MWMT--- Morris water maze test

mTOR ----- Mammalian target of rapamycin

NSQWQ ---- National Standards for Drinking Water Quality-Pakistan
PAK

SOD--------- Superoxide dismutase

TBA--------- Thiobarbituric acid

TCA--------- Trichloroacetic acid

US-EPA----- United States Environmental Protection Agency

WHO-------- World Health Organization

ANOVA----- Analysis of variance

ABSTRACT

Cadmium is known to be associated with etiology of different diseases such as itai-itai disease, hepatocellular injury, neurotoxicity, pulmonary edema, cancer, cardiac and neurodegenerative diseases. It is widely reported that cadmium affects the proper functioning of cells by disrupting the cellular redox balance and hence causes apoptosis. The treatment of cadmium toxicity with medicines bring up unwelcoming side effects so there is a need to safely remove cadmium from the body without any harmful effects. The aim of this study was to determine that how cadmium causing malfunctioning of cells could be treated with avocado and papaya fruit juices. This work was focused on elucidating and comparing the effects of avocado and papaya fruit pulp juice on cadmium dependent impairment in memory and spatial learning, level of biomarkers in hepatic and renal tissues, expression of antioxidant enzymes and level of lipid peroxidation caused by cadmium. In order to develop the Cd rat model, $CdCl_2$ (200ppm) was given to rats in drinking water for 7 weeks. After inducing cadmium toxicity for 7 weeks, post treatment with avocado (10%w/v) and papaya (10%w/v) was administered to rats in standard diet. After completing the cadmium post treatment, memory and learning were assessed via morris water maze behavior test. Biochemical tests for liver and kidney biomarkers was observed by liver and kidney function tests. To determine the level of ROS, lipid peroxidation was determined by MDA assay. Gene expression of SOD, CAT and GPx were determined via qRT-PCR. The results of this study elucidated that avocado and papaya fruit juices were successful in reversing the Cd toxicity. The accumulation of cadmium in liver, kidney and hippocampus tissues was found to be reduced after treatment with avocado and papaya. The gene expression of SOD, CAT & GPX were upregulated in avocado and papaya juice post treatment. Moreover, comparative analysis between avocado and papaya fruit juices elucidated

that papaya has more active potential in improving the memory and learning, upregulating the antioxidant enzymes expression and reducing lipid peroxidation in liver, kidney and hippocampus. This study suggests that diet containing papaya and avocado can be helpful in the treatment of lethal effects caused by cadmium.

CHAPTER 01

INTRODUCTION

More than a few decades ago, the rate of poisoning has risen considerably in view of the abundant availability of multiple chemical products. Individuals can accidentally use certain medicines and chemicals and can deliberately or accidentally be intoxicated. Heavy metals like other poisonous chemicals can pose serious threats to human life from natural or industrial sources. Despite the drastic global manufacturing, consumption and environmental releases of Cd compounds indicate no effective way to recycle them. Cadmium toxicity is one of the world's health issues affecting many organs and can cause fatalities in certain instances annually. Many parts of the world have reported cadmium intoxication. A prolonged cadmium exposure by air, soil, food and water can lead to cancer. Cigarette smoking is the most prominent cause of cadmium hazard to humans. Cd is used commercially in television screens, paints, batteries, lasers, steel galvanizing and cosmetics. Industrial exposure inhalation in the workplace may be important. Welding or soldering, for instance, and serious chemical pneumonitis may occur. Exposure to cadmium is due to ingestion of food or water that is contaminated and may lead to long-term impacts on health. Drug and dietary supplement contamination may also be a contaminating source (Bernhoft *et al*., 2013).

In nature, cadmium is non degradable and therefore will remain in circulation once released into the atmosphere. The U.S. National Toxicology Program & International Agency for Cancer Research have designated cadmium as a human carcinogen evidently, a powerful, multi-tissue animal carcinogen (Waalkes *et al*., 2000). Acute Cd

poisoning is known to cause brain, testes, and liver toxicity, whereas chronic Cd exposure often results in osteoporosis, renal dysfunction, bone fractures or anemia (Abdel Moneim *et al*., 2014; Othman *et al*., 2014). Acute Cd exposure, according to epidemiological and experimental proof, causes oxidative stress due to the antioxidant enzymes inhibition (El-Habit *et al*., 2014).

Public, academic and government interests in traditional or isolated biological medicinal products are seemingly exacerbated by adverse drug responses and the financial burden of the modern medicinal systems. In several traditional schemes millions of individuals have used medicinal crops to treat their diseases. This could be due to the elevated price or absence of belief in orthodox health care or as a consequence of the worldwide change in the use of natural instead of synthetic products. Consequently, as possible treatment and preventive agents, substances with antioxidant characteristics have lately received unprecedented attention (Mohamed Sadek *et al*., 2012).

Antioxidant products, such as ascorbic acid (vitamin C), play an important part in reducing diseases of degeneration and free radicals to maintain chelating reactions. Therefore to check whether the toxic and teratogenic effect generated by heavy metal cadmium is reduced by avocado and papaya fruit pulp that contain a number of antioxidants, the response of cadmium on rats and the ameliorative activity of avocado and papaya fruit pulp in combination with its comparative therapeutic analysis was examined in the following experiment.

1.1. Rationale

The level of heavy metals in drinking water remains unchanged even in the present age of increasing technology, as regulatory agencies have set out in distinct countries of the

globe. The health concerns of government and health practitioners are growing with drinking water contaminated with cadmium. However, the consumption of contaminated drinking water is the predominant origin of human exposure to heavy metals, and lead to cardiovascular diseases, kidney diseases, neuronal damage, and cancer, and diabetes risk. Research has also discovered that intake of heavy metals is one of the factors for increasing infant mortality rates. Important steps are therefore necessary to protect the general public against these health risks. Thus, defensive mechanisms against oxidative harm induced by enhanced ROS levels in the human body resulting from exposure to heavy metals are strongly required (Rehman *et al*., 2018).

1.2. Problem statement and impact

1.2.1. Problem statement

1. Increased usage of Cd in industries causes release of high amount of Cd as a by-product in the environment which is responsible for posing severe human life threats.
2. There is a need to safely detox the body from cadmium mainly by natural sources as medicines have unwelcome side effects.

1.2.2. Impact

1. Production of ROS that causes lipid peroxidation, inhibition of DNA repair mechanism, apoptosis and cell death
2. Induces toxicity in liver, kidney and hippocampus

1.3. Objectives

1. To analyze the effect of *Carica papaya* and *Persea americana* against Cd induced spatial learning and memory impairment in rats
2. To elucidate the effects of *Carica papaya* & *Persea americana* against cadmium induced lipid peroxidation in rats
3. To elucidate and compare the antioxidant effects of *Carica papaya* & *Persea americana* against cadmium toxicity in liver, kidney and hippocampus in rats

CHAPTER 02

LITERATURE REVIEW

2.1. Heavy Metal Toxicity

Metals are basically located in earth's crust in nature. Their concentrations fluctuate with respect to location (Khlifi *et al*., 2010). Heavy metals are called to those metals whose density is more than $5g/cm^3$. In low concentrations, heavy metals are requisite for carrying out foremost physiological functions. Though, heavy metals are adversely toxic in concentrations higher than its threshold concentration for living organisms and environment (Jaishankar *et al*., 2014; Järup *et al*., 2003). These metals displace original metals to bind with the active site of enzymes that causes adverse effects by causing malfunctioning in cells. This eventually causes toxicity known as metal toxicity (Flora *et al*., 2008).

Heavy metals can disrupt the metabolic operations of the body through different methods. Once heavy metals are in the blood, the essential activity in the body is blocked. The heavy metals entry into the atmosphere is due to natural sources and human activities. Heavy metal sources include soil erosion, natural crust weathering, mining, industry effluents, urban rush, wastewater, pest or disease-control equipment, and many other causes.

However, these metals have vital biological roles in animals and plants, they have sometimes been provided an extra advantage by their chemical composition and oxidation-reduction characteristics in order to evade regulation processes such as homeostasis, transportation, compartmentalization and attachment to the necessary cell

structures. These metals attach to protein sites by removing actual metals from their normal binding sites that cause cell dysfunction and eventually toxicity.

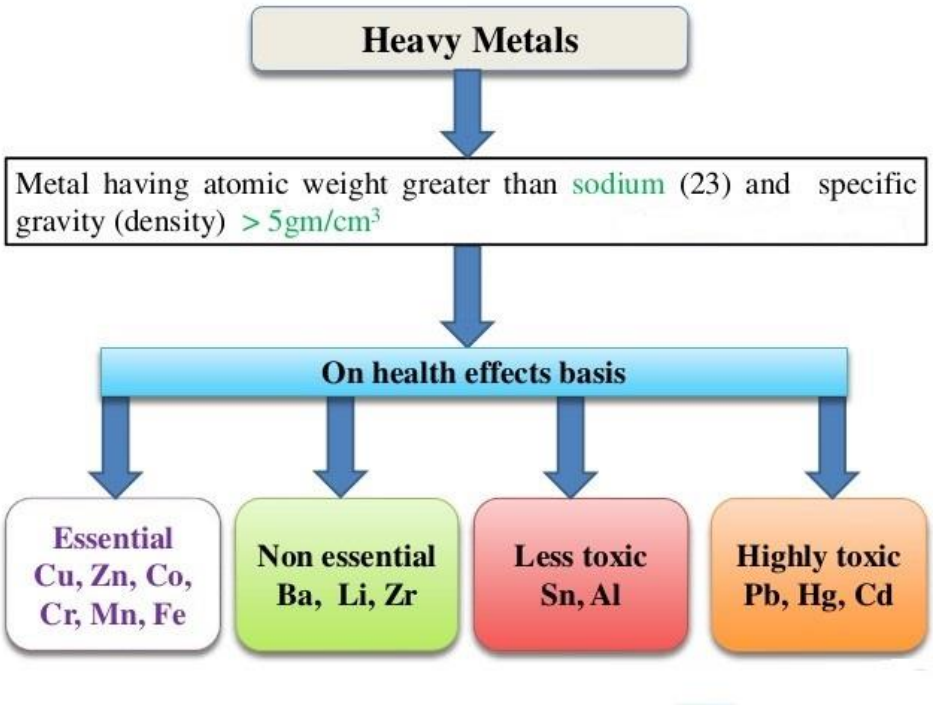

Figure 2.1: Categorization of heavy metals into four groups on health effects basis; essential, non-essential, less toxic and highly toxic (Raikwar *et al.*, 2008).

2.2. Cadmium

Heavy metal cadmium has its concern regarding environment and occupation. Naturally, its average concentration is 0.15mg/kg in earth's crust. As per the ATSDR classification, the seventh toxic heavy metal is cadmium. It is a zinc manufacturing by-product that may be subjected to individuals or livestock at work or in the atmosphere. Once this metal is consumed by animals, it will build up within the body throughout lives.

2.2.1. Exposure and Occurrence

No effective recycling method is available for cadmium although the spectacular manufacturing, usage, and discharge of Cd in the setting are significant globally.

Human exposure to Cd compounds could, therefore, lead to a severe health problem (Munisamy *et al*., 2013).

Japanese endured various rates of emissions after World War II, 1960's and 1970s. One of these circumstances was Itai-Itai disease due to rice fields contaminated with acute cadmium. It was reported that around 400 people were infected by the illness between 1910 and 2007 (Kaji *et al*., 2012). Cadmium is present in marine phosphates and sedimentary rocks in an outrageous concentration of 15mg/kg. It is present in batteries, alloys, and pigments (Paschal *et al*., 2000). About 3/4th of cadmium in the alkaline batteries is used as an electrode, the rest portion is used as a plastic stabilizer in coatings, pigments, and platings. Human beings may be subjected mainly by inhalation and ingestion to this metal and may die from acute and chronic poisoning. For several centuries, cadmium dispersed in the atmosphere remains in groundwater and sediments. These metals are gradually consumed by plants and concentrated along the food chain, eventually entering the human body

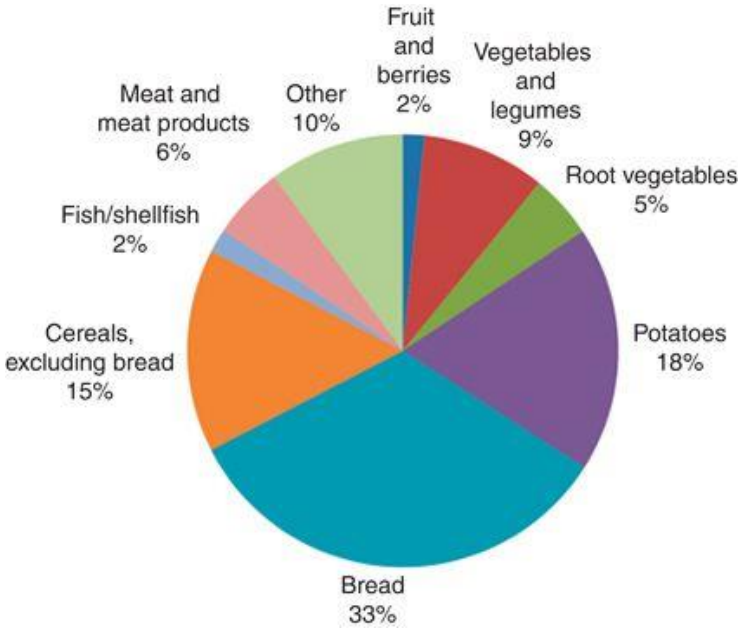

Figure 2.2 Dietary assessment of cadmium exposure in different foods. High level is present in cereals including wheat and rice (Julin *et al*., 2012).

The main exposure sources for human implicates working in metal industries, smoking cigarettes and consuming Cd-contaminated food (Paschal *et al*., 2000). Cadmium is present in some food in trace amounts including potatoes, leafy vegetables, liver and kidney (Satarug *et al*., 2003). Cadmium gets disseminated mainly through the bloodstream (Davison *et al*., 1988). Cadmium causes pulmonary infection, emphysema, and loss of olfactory function on chronic inhalation of cadmium (Mascagni *et al*., 2003).

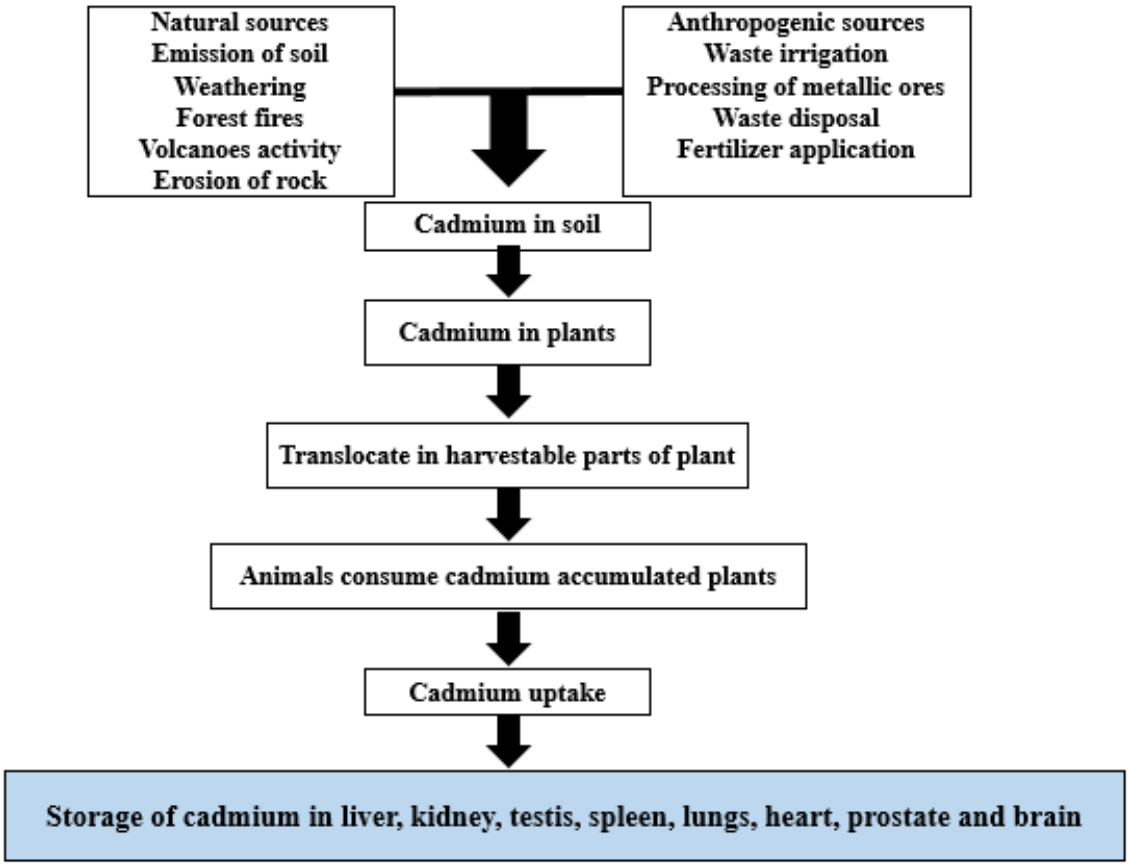

Figure 2.3 Different sources of cadmium exposure. Route for cadmium transportation and storage in plants and toxic effects on consuming cadmium accumulated plants in animals (Verma *et al*., 2017).

The kidney, cardiovascular, liver and reproductive system may be affected by cadmium intoxication, and it can trigger kidney damage, osteomalacia, and lung cancer. Long-term cadmium exposure causes Itai-Itai illness in females primarily and is associated with severe glomerular and tubular structure and generalized osteoporosis and

osteomalacia. This is the reason that sudden infant mortality, ear disorders, asthma, respiratory diseases, lung cancer, and cardiac diseases are at risk in passive smokers (Verma *et al*., 2017).

2.2.2. Epidemiology of Cadmium Toxicity in Pakistan

In Pakistan, the most frequently discovered heavy metals in surface water as well as in groundwater are the arsenic, the cadmium, the chromium, the copper, the nickel and the zinc, which all contribute to safety risk and the atmosphere (Jaishankar *et al*., 2014; M. R. Singh *et al*., 2007).

Drinking water is a fundamental requirement for human survival. Today, there are more than one billion individuals in the globe as a whole. Most of this one billion inhabitants live in urban regions. According to the 1998 World Health Report, water supply has varied extensively for distinct regions and nations. Still, most people in emerging nations, particularly, are vulnerable to terrible conditions as the drinking water carrying heavy metals that are not safe to drink (Baig *et al*., 2012).

Cd was discovered both in the crust of earth and in the seawater in Pakistan. Ocean water has an average Cd of less than 5-110 ng / L, according to IARC (Leung *et al*., 2012) Cd, which is regarded to be allowable for drinking water, is 0.003 mg / L according to the WHO (M. R. Singh *et al*., 2007).

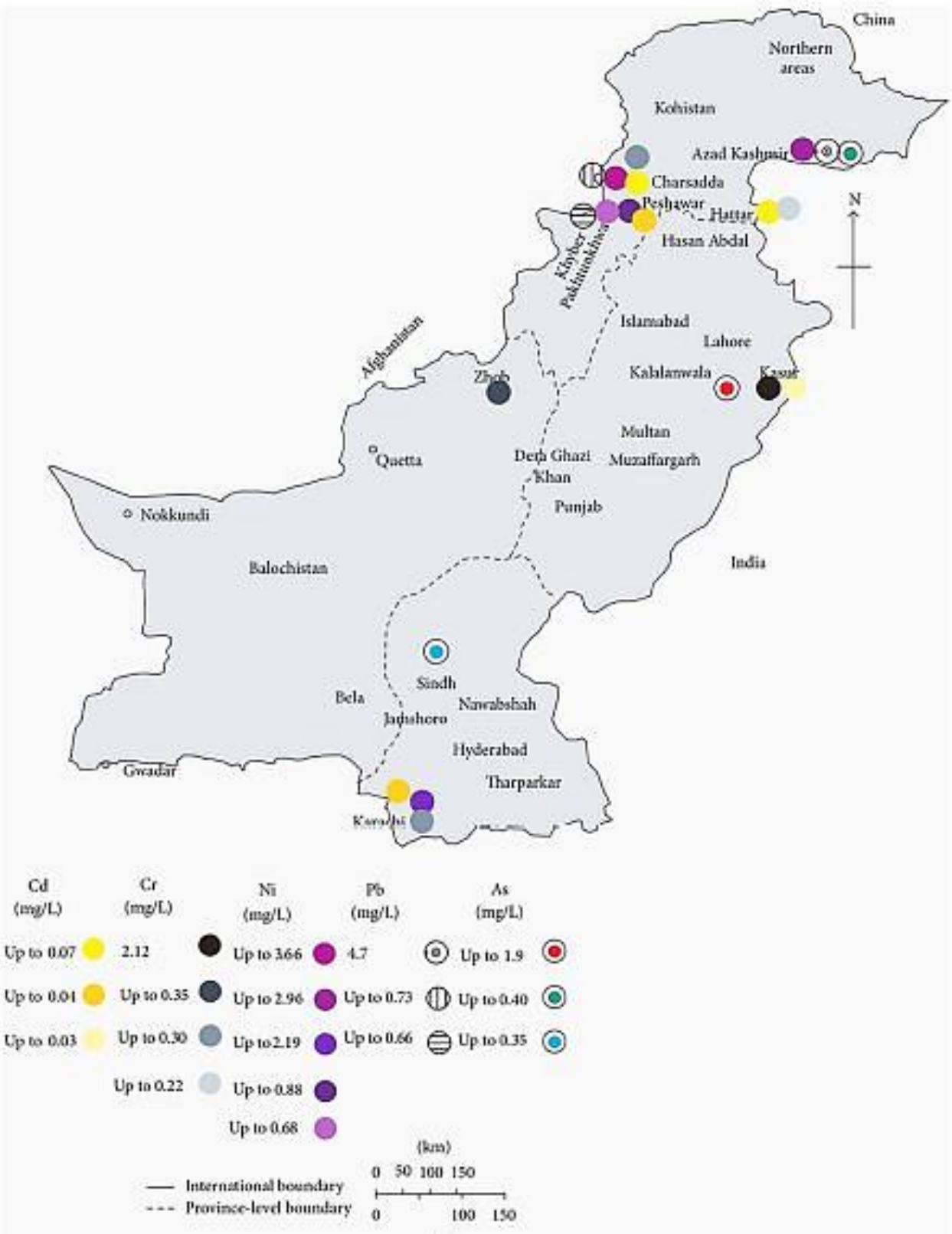

Figure 2.4 In Pakistan map displaying Cr, Pb, Cd, Ni and as groundwater quantity (average numbers; the largest numbers are used where the average value is not accessible). This figure shows Pakistan provinces with the most concentrated heavy metal regions in colored dots, whereas light colors show upper and lower colors with lower density ranges for heavy metals (Rehman *et al*., 2018).

Table 2.1 Allowable constraints in drinking water for heavy metals consumption. Standard threshold values for cadmium consumption set by worldwide research institutes (Rehman *et al*., 2018).

Standards	Cadmium (µg/L)
IARC	Group 1
US-EPA	5
WHO	3
NSDWQ-PAK	10
EU	5.0

A study to evaluate amount of heavy metal in drinking water was performed in Pakistan. According to the study, the Cd level in samples obtained at various areas of Pakistan was discovered to be 0.001-0.21 mg./L (Lone *et al*., 2003). The largest Cd values of approx. 0.02 mg / L were thus discovered in water specimens obtained from Hayatabad Industrial Estate tube well water sources in Khyber Pakhtunkhwa in Pakistan state (Manzoor *et al*., 2006).

2.2.3. Worldwide Epidemiology of Cadmium Toxicity

An evaluation of the quality of groundwater in South-Western Nigeria indicates that Cd and certain other heavy metals were surpassed by the concentrations of WHO in potable water (Adekunle at al., 2007). In Nigeria, Cd-contaminated drinking water was discovered to be 0.06- 1.1 µg / L (Asubiojo *et al*., 1997). Likewise, a drinking water survey in Sweden has resulted in Cd concentrations of up to 5 µg / L (Friberg *et al*., 1979). The average Cd concentration in Saudi Arabia from private wells was 1-26 µg / L (Mustafa *et al*., 1988).

This demonstrates that drinking water is contaminated with Cd in a worldwide manner which may harm people. In the Process readings, we will discuss the main safety issues linked to exposure of Cd.

2.2.4. Cadmium Entry into the Food Chain

The prevalent origin of Cd toxicity are polluted cereal crops, drinking water and food irrigated with polluted water in non-smokers and non-professionally exposed populations (A. Singh *et al*., 2010). Irrigated cereals with wastewater have reduced concentrations of heavy metals in cereals, compared to vegetables.

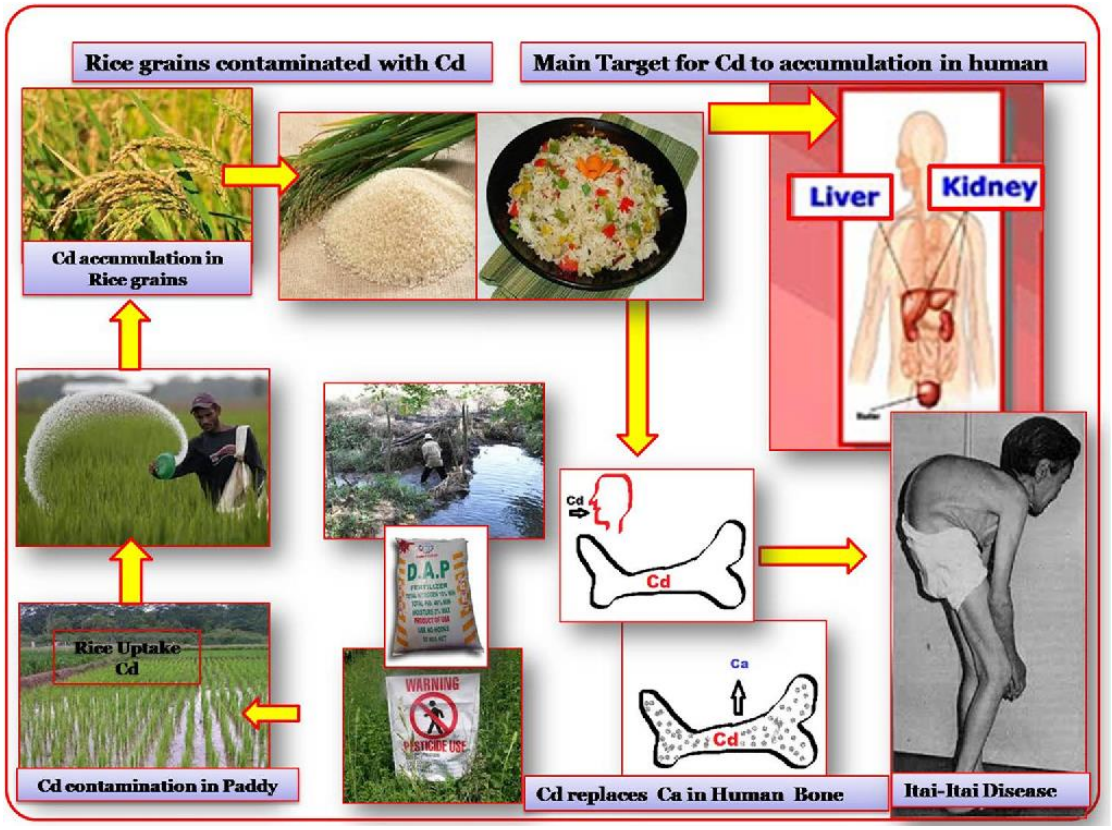

Figure 2.5 Cadmium entry in food chain. Route by which cadmium transported from rice crops to humans. Chronic absorption of cadmium and replaces calcium in bones causing itai-itai disease (Manohar *et al*., 2012)

Figure shows the route by which cadmium enters the food chain. After absorption, the kidney, and other essential organs such as lungs, liver, and bones irrevocably accumulate cadmium (Faroon *et al*., 2012).

Besides its nephrotoxic impact, the renal dysfunction can harm the bone directly or indirectly. The urinary excretions of microproteins including retinol-binding proteins, alpha1-microglobulins, and β2-microglobulins are boosted in kidney damages triggered by cadmium (Bernard *et al*., 2008b).

2.2.5. Absorption of Cd in Body

By various mechanisms Cd may enter the human body. Cd particles (Cd oxides or Cd dichloride) are carried along the main olfactory neurons and accumulated without further migration into the brain in the olfactory bulb. Alternatively, Cd accumulates in the lungs after inhalation and enters blood circulation through the alveolar cells. The other significant mechanism for Cd entry is Cd uptake through ingestion of food or water containing Cd. The proton-metal cotransporter (DMT1) is transporting Cd at the enterocytes apical membrane. Cd exports via the basolateral membrane also involve transporters such as calcium-ATPases and zinc transporters (Branca *et al*., 2018).

When cadmium taken up in the blood, it is mostly transferred to proteins like metallothionein and albumin. Following the intake of the GI blood, the initial organ is the liver. Cadmium here induces metallothionein's manufacturing. Cd-Metallothionine complexes are washed in sinusoidal blood following subsequent hepatocyte necrosis and apoptosis. Part of the absorbed cadmium enter here as cadmium-glutathione conjugates in the bile duct, via secretion, through the entire hepatic cycle. Cadmium reenters the small intestines, which is enzymatically broken down into cadmium cysteine composites in the bile tree.

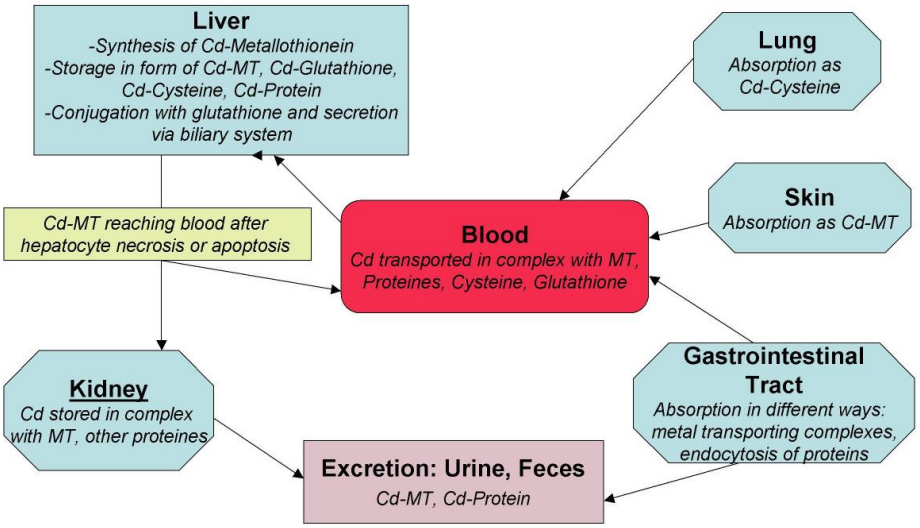

Figure 2.6 Cadmium handling in human body. Transport of cadmium though blood to different organs and excretion out of the body. Effects produce by cadmium in different organs (Godt *et al.*, 2006).

Therefore, a lifetime consumption may lead to cadmium accumulation in the renal, leading to tubular cell necrosis. The kidney is a primary organ for the continual cadmium aggregation (Godt *et al.*, 2006).

2.2.6. Impacts on Health

For a variety of metabolic mechanisms such as peptide hormones synthesis and their action, enzyme metabolism, biomembranes, essential heavy metals are needed. The availability of prerequisite cations for body development is reduced among people at contaminated locations, where there is an increased probability of cadmium absorption, leading to effective bioaugmentation and bioaccumulation in the human body, resulting in detrimental physiological action. Once consumed, cadmium is maintained effectively in the human body and builds up over life (Bernard *et al.*, 2008b). Cadmium is the highly poisonous pollutant and neurodegenerative diseases are caused by its exposure (Chen *et al.*, 2011). Kidneys, particularly proximal tube cells is the principal location for long-term build-up (Bernard *et al.*, 2008a).

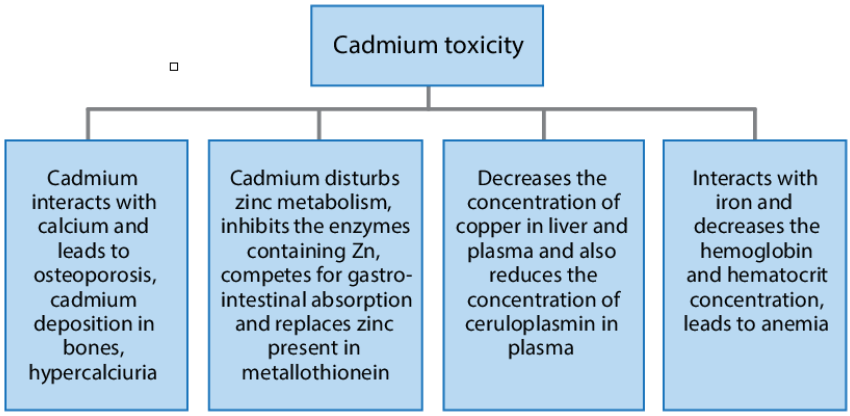

Figure 2.7 Cadmium-induced pathologies in bones, GI tract, liver and kidney. Interaction of Cd with calcium, zinc, copper and iron and the related effects produced by it in respective tissues (Flora *et al*., 2008).

Increased exposure to air-borne cadmium causes impairment of lungs function and the risk of lung cancer among the populations residing in industrial locations or highly polluted atmosphere.

2.2.7. Mechanism for Toxicity

In the human body, toxic metals are metabolized in a similar way as essential elements. The increased consumption of cadmium in the body may be due to a number of reasons such as low intake of Ca, vitamin D, and zinc. The reason for its higher compensating consumption could be it's specific, be relevant molecular resemblance of Cd with Zn and Ca (Godt *et al*., 2006) Cd being non redox active metal and unable to participate in Fenton & Haber reaction, it doesn't produce free radicals (Prasad *et al*., 2008).

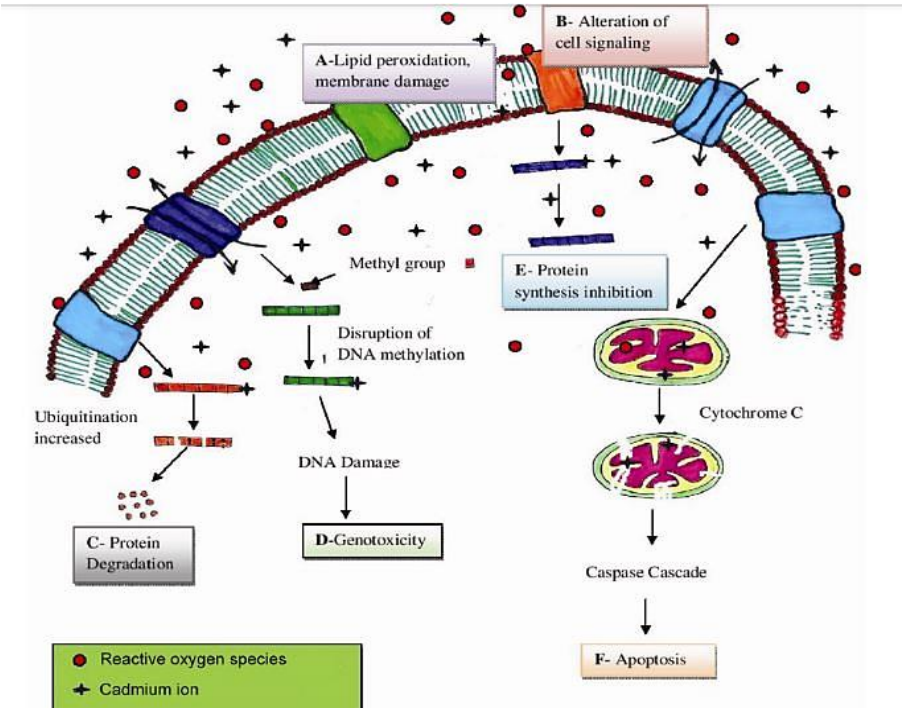

Figure 2.8 Cadmium toxicity mechanisms at the cellular level. (A) Lipid peroxidation (B) Effects on signaling pathway (C) Effects on protein (D) Effects on DNA (E) Effect on synthesis of proteins (Verma *et al*., 2017).

Oxidative stress symptoms such as LPO are the result of (GSH) reduction, as cadmium is linked to GSH and protein chelators derived from GSH (PC) formation. Cadmium affects progression, proliferation, differentiation and apoptotic pathways of the cell cycle. It prohibits replication and repair of DNA. The transporter (DMT1) is a non-specific metal transporter capable of carrying not only iron but also step in intestinal uptake of Cd (Park *et al*., 2002). After Cd entering in the body, the activity of several antioxidant enzymes either reduces or increases (Prasad *et al*., 2008).

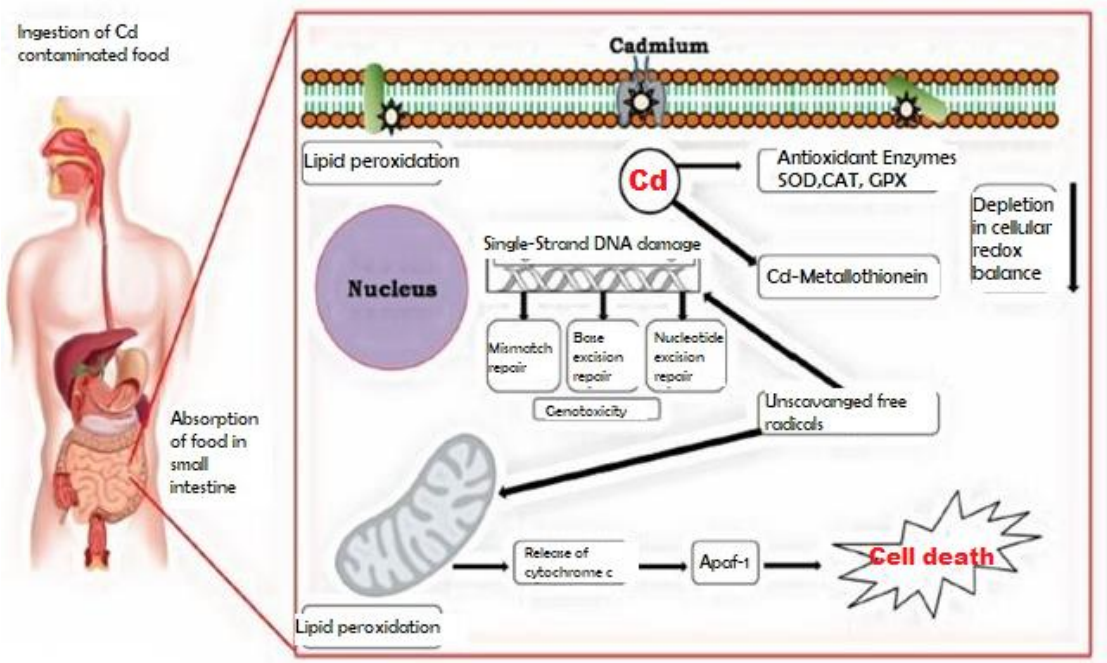

Figure 2.9 Mode of toxicity mechanism of Cd in humans. Intracellular changes caused by chronic exposure of cadmium. Depletion in balance of cellular redox status and cell death by Cd (Kumar *et al*., 2018).

In addition, cadmium causes intracellular ATP concentrations to decline and the mitochondrial membrane eventually breaks down. Ca and cytochrome C concentrations subsequently boost in the matrix of cell and stimulate the triggering of various caspases and induce apoptosis (Méndez-Armenta *et al*., 2007).

Cadmium activates the pathway of (MAPK), through the formation of (ROS), which not only activates the Upstream, Erk1/2 and c-Jun N-terminal kinase (JNK), but also impedes the negative regulatory system, PP2A and PP5 (Protein Phosphate 5) leading to caspase-dependent &-independent neuronal cells apoptosis (Shanker *et al*., 2008).

It also activates the (mTOR) through a ROS induction which is associated with the upregulation of NADPH oxidase (NOX2) expression and its regulatory proteins (Chen *et al*., 2011).

2.2.8. Nephrotoxicity

Cadmium is generally attached to albumin in the circulation after intake and later liver uptakes Cd. Some of this cadmium is bound to groups comprising of thiols such as (GSH) and L-cysteine, which can be consumed by kidney proximally tubular (PT) cells on both its basolateral and apical sides. Cadmium stimulates metallothionein synthesis in the liver, and circulate it in the form of Cd-MT complex. Cd–MT is readily reabsorbed via endocytosis into the PT segment 1 & 2. Multiple carriers carry the free cadmium ions from the blood into the epithelial cells followed by its movement into the urine, in the distinct PT, connected tubular cells and distal convolute tubular (Yang *et al*., 2015).

In PCT cells, the ZIP-8 zinc transporter can also transport Cd and other divalent metals through the apical membrane of the cells. In the intracellular medium of the PCT cells the Cd-MT-1 is stored and breached by lysosomes. Lysosomal DMT-1 will then carry free Cd into the cytoplasm (Y. Liu *et al*., 2001). Protein kinase C activation increases the DMT-1 expression, thus increasing tubular toxicity of Cd (Olivi *et al*., 2001). Free Cd builds up in mitochondria that block complex III of the respiratory chain. The outcome is mitochondrial dysfunction that activates caspase and apoptosis and free radical formation. Free Cd also binds to sulfhydryl protein groups and regulates protein structure and function. Cd has been shown to interfere with the calcium-calmodulin complex's enzyme activity, prevent Na+-K+-ATPase activity and promote MAP kinases (Gunawardana *et al*., 2006; Hirano *et al*., 2005).

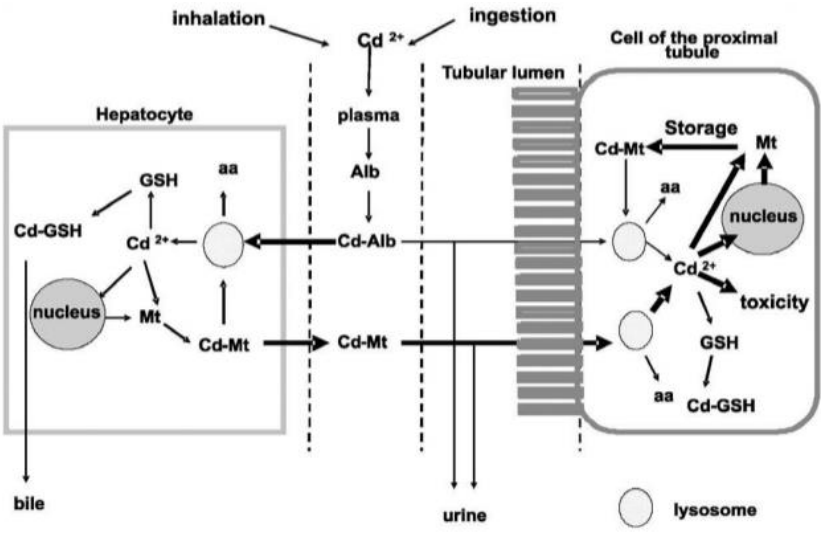

Figure 2.10 Physiopathological mechanisms of cadmium-induced renal toxicity. Route by which cadmium enters from blood followed by accumulation in proximal tubule of kidney. aa: amino acid; MT: metalothionein; Alb: albumin (Bernard *et al*., 2008b).

Cadmium causes nephrotoxicity by generating (ROS), causing apoptosis and inflammation in kidney tissues. The filtration rats of glomerulus decreases on exposure with cadmium hence causes kidney failure (Almeer *et al*., 2019; Ansari *et al*., 2017). The competition for transporter-mediated entering a cell is well established for cadmium with other metals. These transporters focus the most on those for zinc, the cadmium congener and the essential physiological metal ion. The reabsorption of majority of the zinc ions is done by zinc transporters located on the apical side of the renal epithelial cells. In vitro studies have found the role of zinc transporters in the building up of renal cadmium and toxicity. Nevertheless, there is no convincing proof of in vivo characteristics. A previous research showed that pre-treatment of zinc ions decreased the accumulation of renal cadmium and decreased the nephrotoxicity, potentially by removing Cd from the Cd-MT complex. In comparison with the cadmium group, the dietary co-administration of zinc-cadmium chloride reduces accumulation of renal cadmium considerably (Agirdir *et al*., 2002). The possible effects of zinc as a cadmium absorption inhibitor could, however, not be excluded. Indeed, the findings of a different research showed that although the cadmium chloride and zinc chloride co-

injection could considerably decrease the nephrotoxicity induced by cadmium, renal cadmium accumulation remained unchanged (Jacquillet *et al*., 2006). Cadmium gets assembled in the renal cortex on chronic exposure. It primarily affects proximal tubular epithelium leading to aminoaciduria, less reabsorption of phosphates in the renal tubule, glucosuria and β2 microglobulinuria (Goyer *et al*., 1989).

2.2.9. Neurotoxicity

Cadmium causes neurotoxicity leading to neurodegenerative diseases provided proof that $CdCl_2$ increases nerve cell oxidative stress, a consequence that is entirely hindered by exogenous neurotrophic factors. These activities require inhibition of Janus kinases, Jak1 and Jak2 transphosphorylation of the receptors. Jak kinases neuronal inhibition, selectively activated by increasing intracellular oxidative stress levels in neurons, offers a new way of implementing heavy metals that have their neurotoxic effects. Cd also enhances free radical production in adult rat brains and impedes with the antioxidant defense system that causes Cd-induced modification of lipid structure and also harms membrane-bound enzymes such as Na^+ or K^+ ATPase (Shukla *et al*., 1996). The CNS injury generated by Cd seems to be due to a large loss of oxidative phosphorylation along with a range of circumstances causing CNS injury following inhibition of oxidative phosphorylation, all of which deteriorate the white matter selectively in the brain (Fern *et al*., 1996).

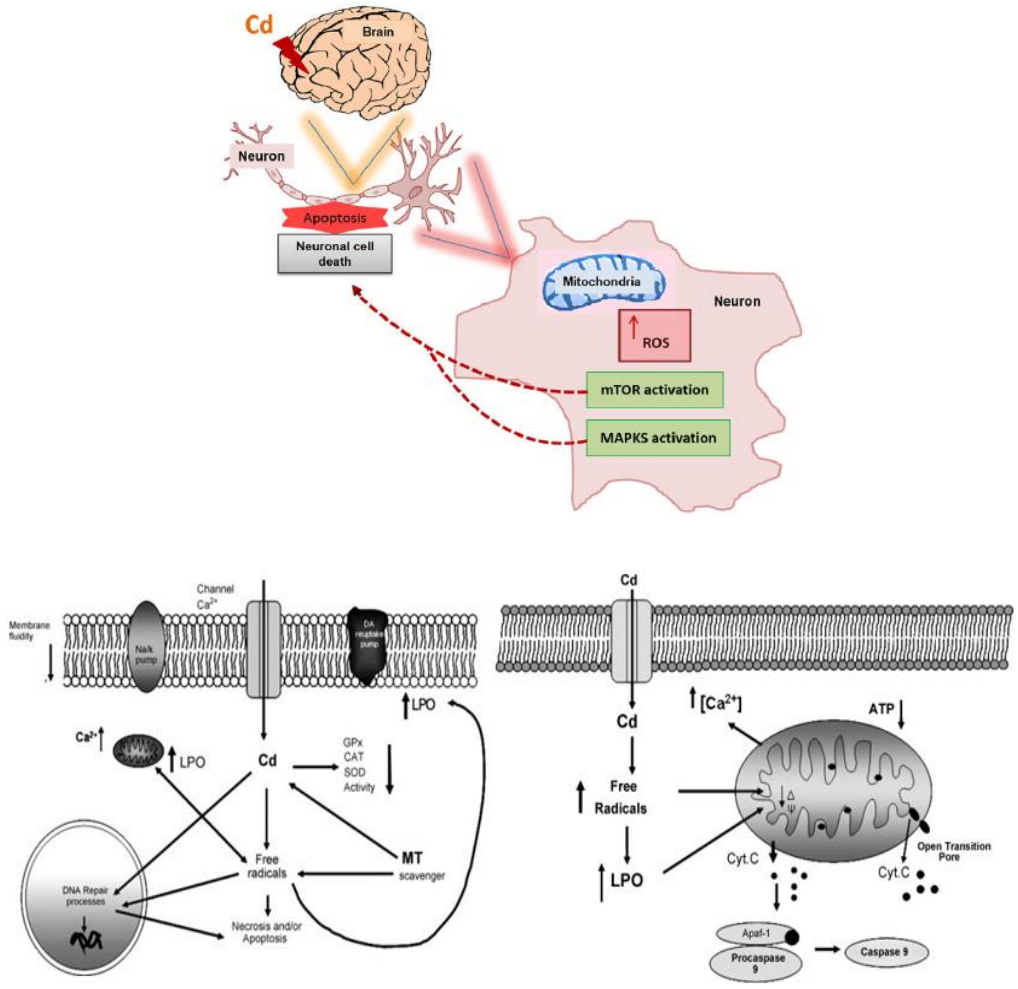

Figure 2.11 Figure depicting neuronal apoptotic cell death following exposure to cadmium. Role of Cd in increasing ROS and activation of MAPK & mTOR pathways leading to neuronal cell death Mechanism of cadmium neurotoxicity. Two pathways in which role of cadmium in causing malfunctioning in nucleus and mitochondria is shown. In both of these pathways Cd takes entry through calcium voltage gated channels and induces apoptosis (Méndez-Armenta *et al*., 2007; Rehman *et al*., 2018)

Cadmium invades neuron through voltage-gated calcium channels (Usai *et al*., 2008). Cd ion enters through Ca^{2+} channels within the neurons. When Cd is inside the cells GPx, CAT and SOD activity significantly reduces hence cause a rise in free radicals. This free production of radicals can produce and enhance lipid peroxidation with cellular membrane disorder and cause apoptosis or necrosis. In addition, Cd and free radical caused harm to DNA, whereas MT is primarily triggered as free radicals and Cd scavengers (Méndez-Armenta *et al*., 2007) . These symptoms include neurological

disturbances, olfactory dysfunction, learning disabilities and mental retardation (Branca *et al*., 2018). The integral part of the brain is hippocampus and it attaches to the limbic system. It is involved in playing its part in spatial navigation. It is present in the medial lobe of the brain. In the case of Alzheimer's disease, hippocampus gets severely damaged (Kante *et al*., 2016) Hippocampus and frontal cortex brain areas are very much involved in memory and learning procedures. In daily memories of facts and habits, these areas play a key role. After prolonged cadmium exposure, altered AChE activities have been previously reported in adult Wistar rat in hippocampus, brain cortex, cerebrum, striatum, and hypothalamus (Batool *et al*., 2017).

2.2.10. Hepatotoxicity

On first exposure with cadmium, the liver is the main organ that absorbs and accumulates a major portion of cadmium (Drug & Toxicol, 2012). Primarily, cadmium binds to a sulfhydryl group on essential molecules in mitochondria. Activation of thiol groups causes oxidative stress, mitochondrial dysfunction and transition in mitochondria permeability.

Secondary injury happens after acute cadmium hepatotoxicity. It deteriorates endothelial cells leading to hepatocellular injury. Thereby, it activates Kupffer cells and infiltration of neutrophils hence activated cascades to induce toxicity (Rikans *et al*., 2000).

After 120 days of daily oral doses of 4.4 mg $CdCl_2$/kg, (Bagchi *et al*., 2000) recorded enhanced hepatic lipid Peroxidation. Zonal, focal and massive necrosis, hepatocyte swelling & fatty infiltration, are the histopathological characteristics of cadmium toxicity in the liver. Inflammation, cirrhosis and fibrosis were also noted, and liver damage often was associated with infiltration of leukocyte (Dudley *et al*., 1984).

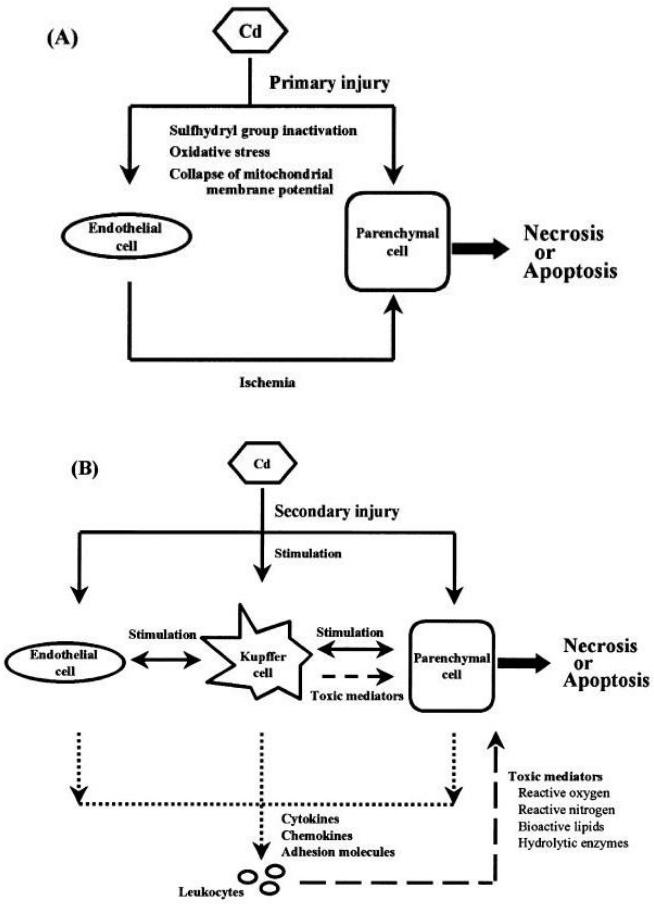

Figure 2.12 Cadmium causing hepatotoxicity (A) Primary injury to hepatocytes (B) Secondary injury to hepatocytes. Both of these pathways leading towards apoptosis or necrosis of hepatocytes (Rikans *et al*., 2000).

2.3. Persea americana (Avocado)

Avocado relates to family 'Lauraceae' that is now being extensively cultivated worldwide. The name' Avocado' was obtained from' ahucatl' in the Aztec language. The alternative names are' Alligator pear' and' Butter Fruit.' The traditional cultivation is based on its high nutritional content and its therapeutic characteristics for food and medicine. The earliest proof of this fruit was discovered in Peru in the 8th century BC, where the seeds of this fruit had been buried with a mummy (Ranade *et al*., 2015) .It is rich in the volatile oil. It cultivates in the warmer region including Central, South and North America. It has medicinal purposes such as healing of wounds, quickens hair growth and treating diarrhea and dysentery. It is cultivated in the Florida, U.S., Hawaii,

and California. Avocado contains terpenoid, alkanols, glycosides, flavonoids, and coumarin. It also contains persin that proves to have a role in inhibiting the generation of superoxide and nitric oxide radicals in cell cultures. It has been reported that different avocado extracts are considered to be anticonvulsant, antioxidant, chrondoprotective, antifungal, insecticidal and antibacterial (Ding *et al*., 2007)

2.3.1. Avocado Composition

Compared to other fruits, the avocado includes a substantial quantity of oil. In Avocado, Lutein is the principal carotenoid. Other carotenoids found in it in small quantities are α-carotene, β-carotene, zeaxanthin, neoxanthin and violetazanthin. In its acetone extracts, tocopherols were also recognized. Lipophilic carotenoids have been reported to have potential anti-cancer effects (Corral-Aguayo *et al*., 2008). Avocado fruits were reported to have hepatoprotective properties because of their flavonoid and phenolic content. The progression in vitro prostate cell lines was inhibited by avocado extract containing carotenoids and tocopherols (Lu *et al*., 2005).

Avocado nutritional supplementation is also very helpful. This is not only because of the nutritional significance of the fruit, but also because of its capacity to improve nutrient intake in other foods (Unlu *et al*., 2018).

2.3.2. Botanical Classification

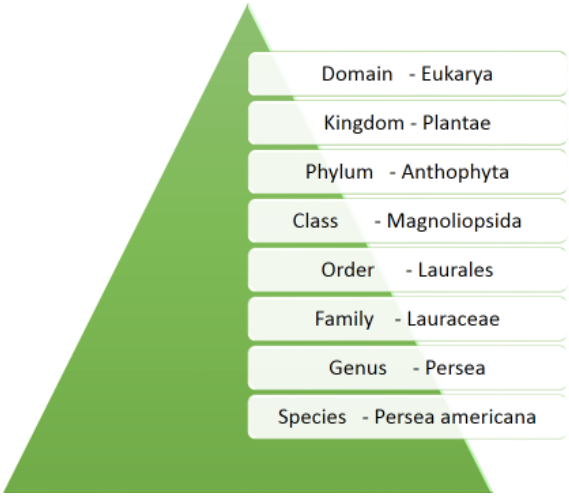

Figure 2.13 Scientific classifications of *Persea americana* (avocado fruit) (Hurtado *et al*., 2018)

2.3.3. Fruit Morphology

Avocado fruit is a berry which is made up by a big core and pericarp seed, which is the sum of the peel, fruit and internal seed (endocarp).

Figure 2.14 The segments of the avocado fruit into seed and pericarp (Hurtado *et al*., 2018)

Ripening is a "extremely coordinated, genetically programmable, and irreversible phenomena" in which the avocado is subjected to major chemical and physiologic changes, such as pulp softening, texture-related changes, color changes (by pigment

synthesis and chlorophyll loss), formation of aroma components, flavor boost and sugar and acid change (Hurtado *et al*., 2018).

2.3.4. Geographical Distribution

Avocado is from Mexico & Central America, where for at least 9000 years it is a staple dietary component. Three distinct ecological breeds can be identified within *P. americana;* Guatemalan, West Indian and Mexican. Avocado cultivars have very different characteristics, such as size, shape, and color (Hurtado *et al*., 2018).

Figure 2.15 External appearances of various cultivars of avocado. 'From left to right, bottom: Ettinger, Fuerte, Pinkerton' top: Gwen, Hass, Reed; (Hurtado *et al*., 2018).

2.3.5. Nutritional Value

Avocado is considered to be very nutritious and now has greater amounts than many other fleshy fruits of insoluble and soluble fiber and proteins. It is also a rich source of potassium and includes vitamins C & E and β carotene. In addition, monounsaturated fatty acids reduce blood levels of unwanted (LDL) effectively, while increasing the beneficial (HDL) (Cowan *et al*., 2015).

2.3.6. Nutrient Composition

Avocado is a nutritious "protecting" fruit, contrary to starch staple products which is characterized as offering "empty calories." Depending on cultivar and growing circumstances, the content of energy for 100 g portion was estimated at 800 kJ.

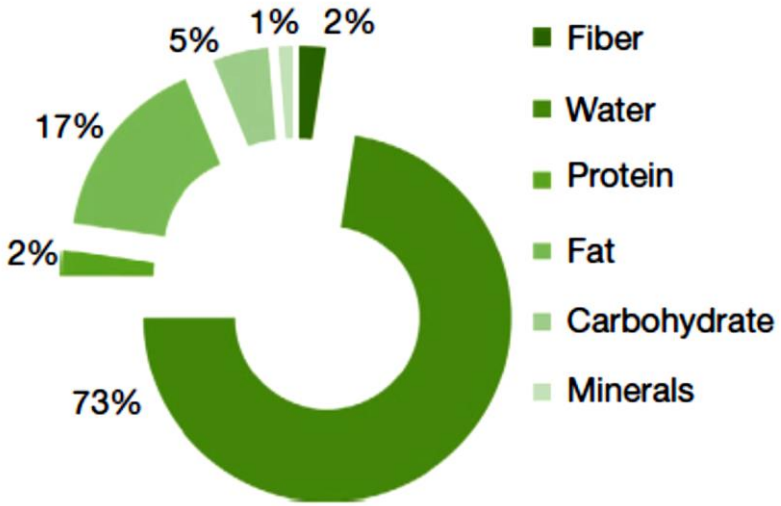

Figure 2.16 Subtropical avocado fruit flesh with nutrient content and composition (G.G, S *et al*., 1975)

The avocado pulp includes roughly 2.3% of fresh-weight protein, two to ten times as much of the other fleshy fruits and vegetables evaluated. While avocados contain all important amino acids, they are often not viewed as protein-dense nutrients. A combination of soluble and insoluble fiber should be optimal in your diet; avocado includes significant amounts of both. Adding avocado to the diet demonstrated a tiny but significant loss of weight. One possible reason is that avocados speed up the human metabolism rate (Nagy *et al*., 1980)

2.3.7. Vitamins & Antioxidants

While vitamin D in avocado has been recognized, no values are reported. The contents of vitamin D in avocados were however revealed to be greater than in eggs and butter. Vitamins A and Vitamins C &E and Provitamin A are thought to act as antioxidants and to prevent' free radicals ' oxygen damage. Oxygen, although vital to life processes,

will also be detrimental when transformed into reactive species of oxygen. They can trigger cell mutations, cancer, arthritis, and heart disease, and can lead to the aging process. The three antioxidant vitamins are efficient for the disarmament of these reactive oxygen species, and the avocado offers approximately twice as much for every three kJ species and for any given daily kJ ratio. In fact, the antioxidant activity in early fruit harvested after 35 days storage is significantly greater than in late fruit harvested after 21 days storage.

Table 2.2 Bioactive compounds present in *Persea americana* fruit pulp. Amount of vitamins and phytochemicals per 100g present in avocado ("USDA Food Composition Databases," ; Vinha *et al*., 2013)

Vitamins & phytochemicals	
Nutrient/phytochemical	**Value per 100 g**
Vitamin C	13 mg
Thiamine	0.61 mg
Riboflavin	29 mg
Niacin	54 mg
Pantothenic acid	507 mg
Vitamin B-6	8 mg
Folate food	0.68 µg
Choline total	0.17 mg
Betaine	0.15 mg
Vitamin B-12	0.4 µg

2.3.8. Mineral Composition

Avocados are a powerful potassium source that is designed to protect people from the danger of strokes and alleviate strokes by up to 40%. One of the perplexing factors causing strokes is the higher blood pressure, which is linked to high sodium intakes.

Table 2.3 Avocado fruit flesh mineral content and composition. The values are given in mg per 100g of fruit (Hurtado *et al*., 2018; "USDA Food Composition Databases,")

Minerals	
Nutrient/phytochemical	**Value per 100 g (mg)**
Calcium	13
Iron	0.61
Magnesium	29
Phosphorus	54
Potassium	507
Sodium	8
Zinc	0.68
Copper	0.17
Manganese	0.15
Selenium	0.4

Avocados are as low in sodium as potassium is present in it. The abundant minerals in avocados are phosphorus, calcium, and magnesium.

2.3.9. Carbohydrates

Studies from the 1940s showed that avocado ingestion may lead to sugar in the urine. D-manno-heptulose, which is the leading reducing sugar in avocado may cause hyperglycemia in humans and other animals (Hurtado *et al*., 2018; X. Liu *et al*., 1999).

Table 2. 4 Sugar content of fruit flesh in a ripe avocado. The concentration range is given in mg/100g of fresh fruit (Hurtado *et al*., 2018)

Sugar (mg per 100g)	Concentration range
Glucose	0.14-1.28
Fructose	0.08-0.65
D-mannose-heptulose	0-3.82
Perseitol	0-2.06

2.3.10. Antioxidant Activity

Avocados exhibit highest lipophilic antioxidant potential in the fruit, which can help to decrease serum lipid peroxidation and enhance vascular health. The compounds Persenone A and B, with distinctive antioxidant characteristics in avocado fruit, have been isolated and proven by Kim *et al*. A possible agent against liver diseases and other oxidative stress related pathologies is the antioxidants activity shown by the methanolized leaf extract of *P. americana* and its hepatoprotective actions against severe paracetamol toxicity.

2.3.11. Hepatoprotective Property

Another group of scientists showed that avocado works on liver. A research on antihepatotoxic activity in rats was carried out by (Ekor *et al*., 2013). *P. americana' s* methanol extract might be protective from acute paracetamol poisoning toxicity and oxidative stress. This protection mechanism is likely be due to the predominant mechanisms of intracellular defense in order to deal with enhanced oxidative stress.

2.4. *Carica papaya*

Carica papaya of the Caricaceae family is regarded in English as the papaya, in Hindi/Urdu as Papita. It originates from tropical America. The plant is recognized for its slender, generally non-connected, smooth stalk, which produces abundant yellow latex and is crowded by a top-cluster of big, lengthy, stemming leaves. The leaves are conventionally used for the treatment of a wide variety of diseases, such as malaria, dengue, jaundice, immunomodulation and antiviral activity (Anjum *et al*., 2013).

Papaya (*Carica papaya*) is produced over 6.8 million tons Worldwide (Nakasone *et al*., 1998). Its' rapid growth is in the largest world industry located in Brazil. It is rich in antioxidants vitamin A, E and C. It also contains vitamin B, pantothenic acid, folate,

and fibers. Moreover, it contains papain and chymopapain that treats allergies, sports injuries and trauma. All the components present in papaya has been proved to be effective against cardiovascular diseases, heart attacks, colon cancer, and strokes. Beta-carotene which is present in higher amount in papaya prevents oxidative damage (Bhowmik *et al*., 2013). It also lowers the level of cholesterol as it is rich in fiber. Being a valuable nutraceutical fruit, papaya has antiviral, antifungal and antibacterial properties (Vij *et al*., 2015).

2.4.1. Carica papaya Linn's Synonyms

International *Carica papaya* synonyms and distinct *Carica papaya* Linn species are described in the table shown below.

Table 2.5 International synonyms for *Carica papaya*. Different names are used by different countries for papaya (Yogiraj *et al*., 2014)

Country	Names
India	Papita
Holland	Tree melon
France	Papaya
Australia	Paw paw
Brazil	Mamao

2.4.2. Botanical Characterization of Fruit

Figure 2.17 Botanical classifications of *Carica papaya* (Gunde *et al*., 2016).

2.4.3. Morphology of Carica papaya Fruit

The fruit is oval and sometimes is called pepo, as it is like melon, with a central seed cavity. On the main stem, fruits are born axillary, generally individually, but occasionally in tiny groups. Fruit weighs between 0.5 and 20 lbs and is green, as opposed to ripe, turning yellow and orange.

Figure 2.18 *Carica papaya* plant profile (Vij *et al*., 2015)

The portion of the edible food is around the large seed cavity. Individual fruits mature within five to nine months, depending on growers and temperature. In 6-12 months, plants start to bear fruit.

2.4.4. Geographical Distribution

Although it is not known the precise region of origin, the papaya is considered to be originally from Tropical America, perhaps in southern Mexico and nearby Central America. Apart from the extensive yet small scale production in Latin America and South Africa, today's successful company manufacturing is mainly in, tropical Africa, Malaysia, Australia, Hawaii Philippines, India, and Ceylon (Yogiraj *et al*., 2014).

2.4.5. Nutritional Value

Papaya is a common, reasonably priced man's fruit that has a high nutritional value. It is rich in natural vitamins and minerals and low in calories. The fruit is a rich source for various enzyme types. Papain, the well-known unruly vegetable pepsin is an outstanding digestive aid that helps to digest the protein in alkaline, neutral and acid food. The Papaya fermented fruit is an advantageous antioxidant nutraceutical. The Papaya lipase enzyme is regarded as "naturally immobilized" as a biocatalyst. It's strongly linked to the water-insoluble portion of crude papain (Marotta *et al*., 2006)

Table 2.6 *Carica papaya* Linn nutritional value per 100 gm fruit. Different constituents in ripe and green papaya is shown (Krishna *et al*., 2008).

Constituents	Ripe papaya	Green papaya
Protein	0.6 g	0.7 g
Minerals	0.5 g	0.5 g
Fiber	0.8 g	0.9 g
Fat	0.1 g	0.2 g
Carbohydrates	7.2 g	5.7 g
Energy	32 Kcal	27 Kcal
Total	2,740 µm	-

2.4.6. Chemical Composition

Papaya is an ordinary, reasonably priced man fruit with a high nutritional value. The calories and natural vitamins and minerals are low. Papaya has low carotene in contrast with other fruits, including plantains, guavas, and apples, which help to block free radicals from damaging them (Yogiraj *et al*., 2014).

Table 2.7 Different constituents present in fruit part of *Carica papaya*. The constituents are given in USDA data base (Saeed *et al*., 2014)

Part of plant	Constituents
Fruit	Protein, fat, fiber, carbohydrates, minerals, calcium, phosphorous, iron, vitamin C, thiamine, riboflavin, niacin, caroxene, amino acid, citric acids Volatile compounds: linalol, benzylisothiocyanate, cis & trans 2,6-dimethyl-3,6 epoxy-7-octen-2-ol Alkaloid: α-carpaine, benzyl-β-d-glucoside, 2-phenylethyl-β-D-glucoside, 4-hydroxyl-phenyl-2-ethyl-B-D glucoside and fur isomeric benzyl-β-D-glucosides

These papaya nutrients help to prevent cholesterol oxidation. Papaya fruit is rich in nutrients such as carotenoids provitamin A, lycopene, vitamin C, vitamins B, nutrient, and fiber supplements. The phytoalexin discovered in the papaya fruit is Danielone. *Colletotrichum gloesporioides*, a pathogenic papaya fungus, exhibited highly antifungal action.

Table 2.8 Minerals present in *Carica papaya* fruit pulp. The values are given per 100g fruit (Pinnamaneni *et al*., 2017; Rivera-Pastrana *et al*., 2010).

Minerals	
Nutrient/phytochemical	**Value per 100 g (mg/µg)**
Calcium	10
Iron	0.25
Magnesium	21
Phosphorus	10
Potassium	182
Sodium	8
Zinc	0.08
Copper	0.045
Manganese	0.04
Selenium	0.6 µg

2.4.7. Vitamins & Antioxidants

Papaya leaves, seeds, and juices have a free radical and antioxidants activity. Papaya juice is an efficacious hydroxyl radical's scavenger (OH^-), which considerably reduces the level of lipid peroxidation and increases the antioxidant activity in rodents. Papaya is an excellent source of vitamin A, vitamin C and dietary fibers as well as a solid vitamin E source (Krishna *et al*., 2008).

Table 2.9 Vitamin content and phytochemical in fruit flesh of *Carica papaya*. The values are given per 100g of fruit (Krishna *et al*., 2008).

Vitamins & phytochemicals	
Nutrient/phytochemical	**Value per 100 g**
Vitamin C, total ascorbic acid	60.9 mg
Thiamine	0.023 mg
Riboflavin	0.027 mg
Niacin	0.3 mg
Pantothenic acid	0.19 mg
Vitamin B-6	0.038 mg
Folate food	37 µg
Choline total	6.1 mg

Phytochemicals	
Nutrient/phytochemical	**Medicinal purpose**
Glucosinolates	Apoptosis, cancer
Papain	Cancer coating
Chlorogenic acid	Diabetes, Cancer, ROS
Caffeic acid	ROS
Kaempferol	Cancer, ROS

2.4.8. Antioxidant activity

Methanol extract from *Carica papaya* unripe fruits was assessed in vivo for its effect on certain antioxidant enzymes that include GPx and GST in mice treated with an oral dose of 100mg / kg. GR, GST, GPx and the glucose-6-phosphate dehydrogenase activity are significantly increased owing to the fraction of the ethyl acetate. After administration of ethyl acetate fraction, significant decreases in the GPx were noted in the kidneys. Quercetin and β-sitosterol have been proposed to account for the potential of antioxidant.

Acrylamide has caused many adverse effects of the tissues as a result of substantial increase in lipid peroxidation, decreased glutathione and reduced catalase and SOD dismutase activities. Administered alone or in combination with acrylamide, the papaya fruit aqueous extract significantly lowers the lipid peroxidation, enhanced glutathione levels, catalase activity, and superoxide dismutase, and strengthens the immune condition expressed in increased IgG and IgM (Mohamed Sadek *et al*., 2012). The papaya juice is secure and has antioxidant compounds that are similar to the norm of a tocopherol. The largest concentration of antioxidants (80%) at 17.6 mg / ml concentration was shown in vitro evaluation of papaya antioxidant impacts(Mehdipour *et al*., 2006). After all papaya juice doses (100%, 200%, 400 mg / kg / day) were taken in blood, blood peroxidation decreased considerably to 35.5%, 39.5% and 40.86% compared to 28.8% for vitamin E respectively. Total antioxidant blood power has increased substantially by 11.11%, 23.58% and 23.14% respectively in papaya juice (100,200,400 mg / kg / day). Vitamin E had a value of 18.44% (Lim *et al*., 2012).

2.4.9. Hepatoprotective property

The dried aqueous and ethanol extract from *Carica Papaya* for its hepatoprotective action against CCl_4 induced hepatotoxicity in rats has been found. Significantly reduced biochemical parameters such as SGOT, serum bilirubin, SGTP, alkaline Phosphatase have seen with significant aqueous extracts (250mg / kg BW) and ethanol (250mg / kg BW) *(*Sadeque *et al*., 2010).

2.4.10. Neuroprotective property

Scientists discovered that the fermented yeast papaya preparation, FPP, has antioxidant activities and can be a successful prophylactic food for free radicals such as cancer, diabetes and particularly for neurological disorders including Parkinson's disease or

Alzheimer's, linked to age, and neurological diseases (Imao *et al*., 1998). The deposition of β-amyloid (ABTA) was linked in pathogenesis of (AD). Studies discovered that (FPP) had the ability to attenuate β-amyloid precursor protein (Zhang *et al*., 2006).

2.4.11. Nephroprotective Property

Studies showed that the serum concentrations of creatinine, urea and uric acid, increased significantly by intraperitoneal injection of CCl_4 and the histologically important characteristics of serious interstitial tubular necrosis in rats. However, in rats pre-treated with the graded oral doses of the aqueous *Carica papaya* (CPE) seed extract, variations in measured biochemical parameters were considerably reduced, in a dose-related way. The extract at 400 mg / kg / day CPE, lasting up to 3 hours after CCl_4 exposure, offered the maximum nephro-protection. The research has found the impact of CPE on CCl_4 kidney-injured rats that can be mediated via either an antioxidant and/or a free radical mechanism (Adeneye *et al*., 2009).

CHAPTER 03

METHODOLOGY

3.1. Chemicals

Cadmium chloride, a white anhydrous compound, odorless, colorless, highly soluble in water with vapor pressure 10mmHg and vapor density 6.3 vs air, highly toxic, carcinogenic and toxic for environment; TBA ($C_4H_4N_2O_2S$), TCA ($C_2HCl_3O_2$) were all purchased from Sigma-Aldrich, USA.

3.2. Animals

Experiments on lab animals were carried out in agreement with regulations by the Institute of Laboratory Animal Research, Division of the Earth and Life Sciences, NIH,USA (Care, Animals, & Resources, 1985). Approval has been granted from the International Review Board (IRB), ASAB, NUST.

Twenty Wistar rats (200-250 BW) were used in this study. The animals have received food and water *ad libitum* at 22–25^{o}C at 12:12 hours of a light-dark cycle with 30% raw protein, 9% crude fat, 4% crude fiber, and 10% moisture content.

3.3. Preparation of avocado and papaya fruit pulp juice

Avocado (*Persea americana*) and Papaya (*Carica papaya*) were obtained from Metro, Islamabad city, Pakistan. Fruits were washed properly and peeled out. The seeds were discarded to obtain the succulent part of the fruit. Afterward, Papaya and avocado was homogenized in a blender using water 500g/500ml without sugar. Specifically, only fruit pulps were homogenized in a blender. After that fruit juices were filtered and stored in a clean container at -20^{o}C.

3.4. Experimental design

In order to evaluate the effect of Cd on hippocampus, liver and kidney in-vivo and its treatment with *Carica papaya* and *Persea americana* fruit pulp in-vivo, experiments were performed. The animals were split up into four equal groups of five rats. The other three groups were received 200ppm $CdCl_2$ in drinking water for seven weeks to induce toxicity in the liver, kidney, and hippocampus (Bernard *et al*., 1980; Casalino *et al*., 1997; Mitsumori *et al*., 1998). $CdCl_2$ was given to animals for 7 weeks to build Cd rat models and distilled water was provided to the control group for the same time period. Afterwards avocado and pulp fruit juices (10% w/v) was given to Cd rat models. Behavior tests were carried out to determine spatial learning and memory. Animals were dissected after behavior tests, blood was collected for biochemical parameters (ALT, ALP, total bilirubin, creatinine and urea) followed by collection of tissues (hippocampus, liver & kidney). The tissues were used for gene expression studies of antioxidant enzymes SOD, CAT and GPx.

The animals were marked in four groups as follows:

- **Group A**: Distilled water was given to rats orally and standard diet (Control group).
- **Group B:** Rats were given with 200ppm $CdCl_2$ in distilled water for seven weeks (Cd intoxicated group) with standard diet.
- **Group C:** Rats were given with 200ppm $CdCl_2$ in distilled water for seven weeks to induce toxicity. After this period, 10% w/v *Persea americana* fruit pulp juice mixed with standard diet (w/v) was given for five weeks to Cd rat model (*Persea americana*-Cd group).
- **Group D**: Rats were given with 200ppm $CdCl_2$ in distilled water for seven weeks to induce toxicity. After this period, 10% w/v *Carica papaya* fruit pulp juice mixed

with standard diet (w/v) was given for five weeks to Cd rat model (*Carica papaya*-Cd group).

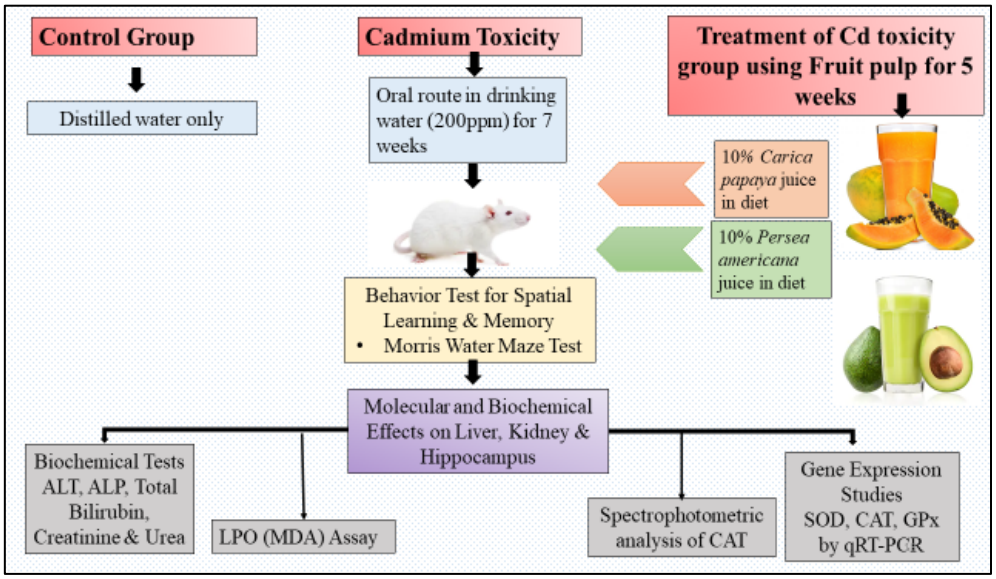

Figure 3.1 : Experimental design for induction of Cd toxicity and post treatment of Cd rat model with 10% w/v avocado and papaya juice mixed with standard diet.

3.5. In vivo experiments

3.5.1. Administration of $CdCl_2$

The rats were administered with 200 ppm $CdCl_2$ for 7 weeks in drinking water and distilled water was given to control for the same time period.

3.5.2. Behavioral assay

The behavioral test was conducted in order to evaluate how Cd intoxication and its treatment can affect memory and spatial learning. Five rats from each group were used in this study after finishing the Cd intoxicated group dose and Cd-avocado/papaya group dose. The behavior tests have been conducted between 10 a.m. and 5 p.m., fifteen minutes previously, mice had been moved to the behavioral test room to allow them to comprehend the circumstances of the behavioral test room. The animals were placed in a space (25^{o}C) reserved for this purpose for the experiment. The same experimenter

performed this experiment. The behavior tests were performed without an experimenter and all tests were recorded with a video camera and analyzed later.

3.5.2.1. Morris water maze test

The MWMT was conducted to establish the animal's spatial learning and memory. As earlier mentioned, the test procedure was conducted with certain changes. The device consisted of a tank of round steel with a diameter of 120 cm and 60 cm deep. The swimming pool was divided into 4 quadrants: North (N), South (S), East (E), and West (W) (Bromley-Brits *et al*., 2011). A translucent platform, 13 cm in diameter and 32 cm in height, in the south-west quadrant was put to escape animals from swimming in water. Spatial indicators were laid on the walls of the pool for the animals to navigate around the perimeter to find a hidden platform. The pool was filled with opaque water up to 2 cm above the platform that makes the platform totally submerged and invisible for the animal.

- Every day for five days a total of five tests were conducted.
- Every day, i.e. Southwest (SW), the platform was located at the same place for 5 days but the release location of the rat for each test was changed. In each trial the rat could examine the platform for 60 seconds and a 10-minute interval was allowed. The rat was permitted to remain on the platform for 5 seconds before cut-off time (60 seconds) and then moved to its home cage after drying. If it did not discover the platform in 60 seconds, though, the rat could remain there for 20 seconds before returning to its house box on the platform by experimenters.
- The average time taken by the rat was registered and the escape latency of the animal for the day was taken on average five tests. Subsequently, the probe test

was done to remove the platform and maintain the animal release point i.e. West (W).

- The rat's reference memory was monitored by calculating the time the animal had spent on the platform in the quadrant. The number of crossing over the last platform place was also determined.

Table 3.1 Direction of release of animals for MWM test. F (Bromley-Brits *et al.*, 2011).

	DIRECTION OF RELEASE				
Days	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
1st	West	South	North	East	South
2nd	North	West	East	West	South
3rd	North	East	West	South	North
4th	East	South	West	East	North
5th	West	South	North	East	South
6th	SINGLE TRIAL WITHOUT PLATFORM. RELEASE DIRECTION, * WEST				

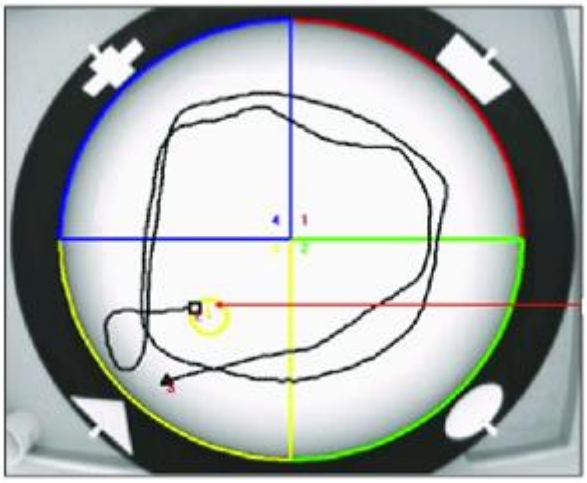

Figure 3.2 Morris water maze (MWM) apparatus. There are four quadrants with different spatial cues. Red line indicates the platform for rat escape in MWM test

3.6. Blood sample collection

Cardiac punching is an ideal method for a single, significant, high-quality sample of a euthanized rat. The rats were anesthetized by means of chloroform. The rat was placed on its back. Left index finger was placed at the lower lib level. The heart was located 1cm above this point. The syringe was held at 45 degree angle. The needle was inserted and a drop of blood was watched to come into the needle. The plunger was pulled to fill the syringe without moving syringe from its place. The needle was carefully disconnected after taking blood from heart in syringe and collected it into serum separating vial. Then the blood samples were centrifuged at 3500rpm for 10 minutes in a centrifuge to separate the serum.

3.6.1. Serum biochemical analyses

Biomarkers for liver and kidney function test such as alanine aminotransferase, alkaline phosphatase, total bilirubin, creatinine and urea of blood samples taken from different groups were analyzed by using Merck kit and Microlab 300-Chemistry Analyzer.

3.7. Tissue sample preparation

After anesthetization by chloroform, animals were sacrificed. Liver, kidney, and hippocampus were immediately removed, placed in ice-cold isotonic sucrose and stored in 10% buffered formalin at 4^{o}C. Later the liver, kidney and hippocampus regions were taken and minced into small pieces, then homogenized with ten volumes of phosphate buffer (0.1mol/L, pH 7.4). It was then sonicated for a total run of 5 min (10s run and 10s pause), 1 cycle and 100% amplitude by using Hielscher Ultrasound technology. The homogenate was centrifuged at 4000 rpm for 10 minutes at 4^{o}C and the clear supernatant collected and stored at -20^{o}C until assays.

Table 3.2 Recipe for 10% neutral buffered formalin (100ml)

Sr.No.	Reagents	Amount
1.	37% formaldehyde solution	10 ml
2.	Potassium phosphate monobasic	0.4 g
3.	Potassium phosphate dibasic	0.65 g
4.	Distilled water	90 ml

3.8. Lipid peroxidation assay

MDA as a marker of LPO was determined according to the double heating method (Draper *et al*., 1990).

3.8.1. Principle

The principle of this method is the quantification of color produced during the reaction of (TBA) with MDA by spectrophotometer. Lipid peroxidation is an oxidative lipid degradation. This process involves free radicals taking electrons (usually in cell membranes) from the lipids and leading to cell damage. To evaluate oxidative stress, quantification of lipid peroxidation is crucial. Lipid peroxidation constitutes natural bi-products of reactive aldehydes like (MDA) and 4-hydroxynonenal (4-HNE). One of the most commonly accepted assays for oxidative damage is the measurement of lipid peroxidation products.

3.8.2. Protocol

In short, 2.5 ml of 10% TCA has been added to 0.5mL homogeneous tissue and put for 15 minutes in a boiling water bath. The mixture was centrifuged at 1000 rpm for 10 minutes after cooling. The tube comprising 1 ml TBA (w / v) of 0.67 percent was filled

with 2ml of supernatant and stored for 15 minutes in a boiling tub. Then the solution was cooled and absorption measured at 532nm by spectrophotometers MDA concentration was determined using the MDA-TBA complex absorption coefficient $1.56x10^5$ $cm^{-1}M^{-1}$ expressed within μM.

3.9. Spectrophotometric analysis of catalase level

Catalase activity was determined with the Sinha technique by adding 1ml phosphate buffer, 0.1ml tissue homogenates, and 0.4ml hydrogen peroxide (Sinha *et al*., 1972). At room temperature, tubes were incubated for 1 minute, cooled at room temperature. By adding dichromate acetic acid the reaction was halted. The pipes were kept cool and color was read at 620 nm in a boiling water tub for 10 minutes. The activity of catalase was determined by a formula in U / g.

Catalase activity= $Ab_{standard} - Ab_{sample} / Ab_{standard}$ X 1 / g of tissue used per test

3.10. Antioxidant enzyme expression

Quantitative analysis of endogenous antioxidant enzymes such as SOD, CAT, and GPX was carried out.

3.10.1. RNA extraction

In accordance with the protocol supplied by the manufacturer, trireagent was used to extract RNA from liver, hippocampus, and kidney. Before the sacrifice, animals were anesthetized with chloroform. Hippocampus, liver as well as kidney were harvested quickly. The 50-100 mg of the rat tissue was homogenized in 1 ml of tri-reagent by using glass homogenizer. Samples were provided a five-minute stand at room temperature to permit nucleoprotein complexes to fully dissociate. The chloroform was introduced to the samples afterward rigorously shaken for 15 seconds until the mixture

became milky. At room temperature, a mixture was added to a stand of 10 minutes followed by a centrifugation for 15 minutes at 12000xg at 4^{o}C. Centrifugation led to a three-phased mixture separation: lower red with protein, opaque interphase DNA and upper aqueous phase containing colorless RNA. The RNA phase was carefully removed to prevent DNA contamination. Isopropanol (0.5 mL) was mixed with 2-3 times eppendorf inversions in this aqueous stage and was supplied with a stand at room temperature for 10 minutes to reduce the possibility of DNA contamination. This mixture was again centrifuged for 10 minutes at 12,000xg and caused the RNA to precipitate along the eppendorf tube in the form of a white pellet.

After removing supernatant, the RNA pellet was washed with 1ml of 75% ethanol. Ethanol was diluted to inactivate RNase enzyme in DEPC water. The RNA pellet with ethanol was centrifugated for 5 minutes at 7500 rcf (4^{o}C). Samples of RNA were then saved to use at -80^{o}C. It was separated from -80^{o}C to utilize RNA for reverse transcription (RT). The pellet has been re-suspended in 30 µl PCR water, with the removal of 75% ethanol.

The pellet was given 5 minutes of heat shock after re-suspension to ensure that the pellets were completely dissolved and secondary structures were removed from the RNA sample. The spectrophotometer evaluated the RNA concentration quantitatively following a heat shock.

3.10.2. RNA quality

Agarose gel (2%) was used for RNA samples in order to ensure consistency of RNA and to evaluate the quality of authentic results. Only clear, sharp 28S and 18S RNA bands were further processed.

3.10.3. Reverse transcription

The cDNA was synthesized by using 1µl of 10mM oligodT primer along with the introduction of 4µl reverse transcriptase (RT buffer, Thermo Scientific), 10µl of RNA, 2µl of 10mM dNTPs, 0.5µl of RNAse inhibitor, 1µl of RT & Nuclease free water would be added to make the volume up to 20µl. Afterward tubes containing reaction mixture were incubated at 42°C for 1 hour followed by 70°C for 10 minutes.

Table 3.3 Recipe for cDNA Synthesis

Components	Quantity (µl)
10mM oligodT primer	1
RNAse Inhibitor	0.5
10mM dNTPs	2
RT Buffer	4
DEPC Treated Water	1.5
Reverse Transcriptase	1
RNA	10
Total Volume	20

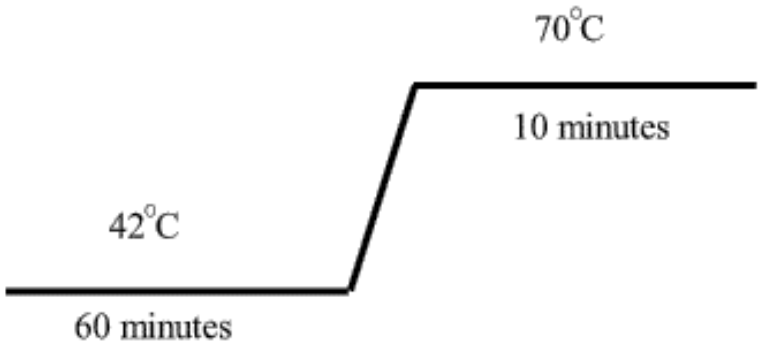

Figure 3.3 RT PCR Cycle

3.10.4. Real-time PCR

Real time PCR was conducted using 25µl reaction mixture containing 12.5µl of 2X SYBR Green/ ROX qRT PCR reaction mix (Thermo scientific), 1µl of each 10mM forward & reverse primers, 1µg cDNA and 8.5µl of water. The amplification program is follows: denaturation at 95^{o}C for 10 minutes, 40 cycles for denaturation at 95^{o}C for 45 minutes, annealing at 60^{o}C for 60 seconds and then extension at 72^{o}C for 45 seconds. Triplicate samples were carried out for each reaction. The quantitative fold changes in the expression of mRNA was determined compared to β actin mRNA levels in each respective group. The primers used for real time PCR are listed in the table given below. The Graphs obtained were analyzed on IQ5 (BioRad).

Table 3.4 The primer sequence with their corresponding melting temperature and GC content of each primer for respective gene used in the study

Gene	Primers (5'⟶ 3')	Melting Temperature (Tm °C)	GC content %
CAT-F CAT-R	TCCGGGATCTTTTTAACGCCATTG TCGAGCACGGTAGGGACAGTTCAC	61.1 68.7	43.5 58.3
SOD-F SOD-R	AGCTGCACCACAGCAAGCAC TCCACCACCCTTAGGGCTGA	62.5 62.5	60 60
GPx-F GPx-R	GGCAAGGTACTACTTATCGAG GTTCACCTCGCACTTCTCGAAG	59.4 64	47.6 54.5
β actin F β actin R	GGCATCCTGACCCTGAAGTA GGGGTGTTGAAGGTCTCAAA	60.5 58.4	55 50

Table 3.5 Recipe for Real time PCR

Components	Quantity (µl)
2X SYBR Green/ ROX qRT PCR Reaction Mix	12.5
10mM Forward Primer	1
10mM Reverse Primer	1
Water	8.5
cDNA	1
Total Volume	25

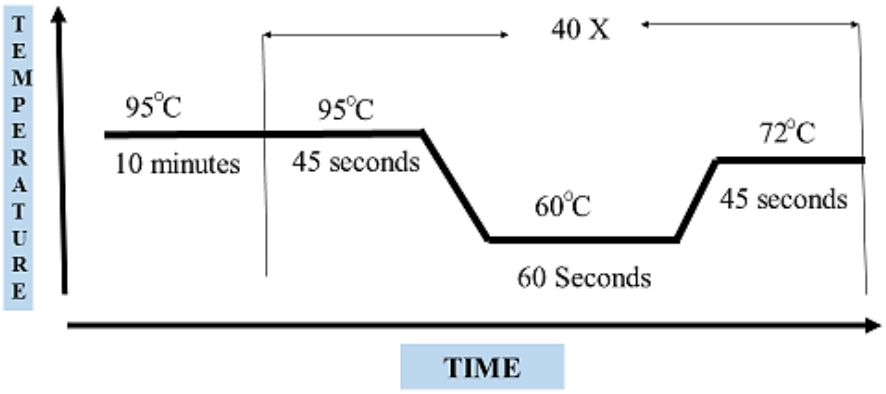

Figure 3.5 Real Time PCR Cycle

3.11. Histopathological studies (Hematoxylin and eosin staining H&E)

H&E is a common histological staining technique. It is the most commonly used medical diagnosis stain.

3.11.1. Principle

The chemical attraction between the tissue and the dye is the principle behind H & E stain. The basic coloration of hematoxylin imparts purple & blue contrast with basophile structures, mainly those that contain nucleic acid moieties such as chromatin, ribosomes and RNA-rich areas. An acidic eosin contains the fundamental components of the pink, orange and red components such as the RBCs, cytoplasm, muscle and collagen.

3.11.2. Protocol

Samples (liver, kidney, and hippocampus) from each group were selected, transversely cut and fixed in 10% formaldehyde solution, then conserved in paraffin. The tissues were deparaffinized by flaming the slide on burner. The treatment was repeated to remove the wax. The xylene was drained and the tissue section was hydrated by passing through decreasing concentrations of alcohol i.e. 100%, 90%, 80% & 70% followed by hydration with water. Afterwards staining was carried out with hematoxylin for 3-5 minutes. The excess dye was selectively removed by immersed the slides in 1% acidic alcohol for few seconds. It was rinsed in running tap water. It was then counterstained with 1% eosin for 10 minutes. It was washed in running tap water and then dehydrated in increasing concentrations of alcohol. The slides were cleared by giving it two xylene baths. The slides were mounted in xylene based mounting media (Kiernan *et al*., 1990). The sections were analyzed under Optika microscope and photographed with a digital camera.

3.12. Statistical analysis

Five rats in every class conveyed data as mean + SEM. One-way ANOVA and two-way ANOVA were used for data comparisons followed by a Tukey and Bonferroni post-hoc exam respectively. The findings were statistically significant at $p < 0.05$.

CHAPTER 04

RESULTS

4.1. Effect of Treatment on Body Weights

The body weights were measured before starting the experiment, after inducing Cd toxicity and after administration of fruit juices in Cd rat model. Initially there was no significant difference in BWs of all experimental animals. The induction of Cd toxicity in rats significantly caused weight loss at the rate of 17.91% as compared to controls. However, treatment with *Carica papaya* significantly ($p<0.001$) filled out rats by 67.29% followed by *Persea americana* per 31.24% compared to $CdCl_2$ treated group.

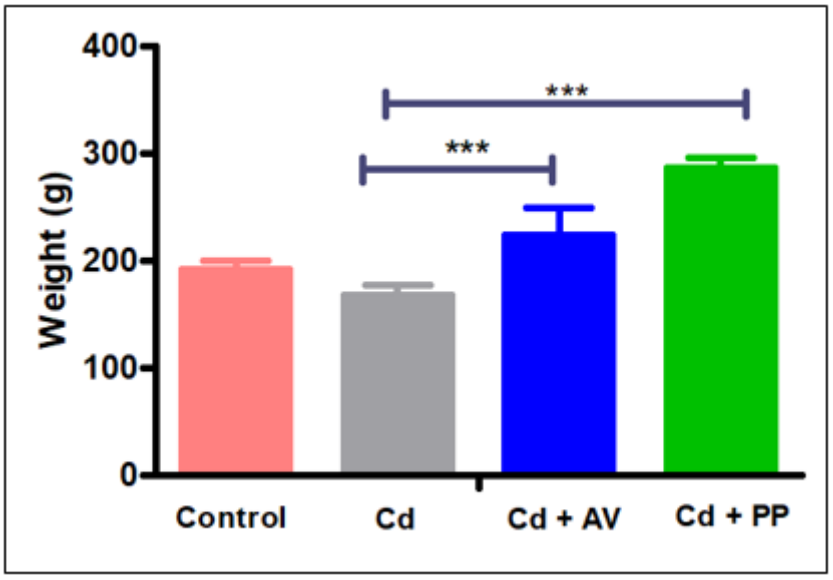

Figure 4.1 The effect of *Persea americana* (Avocado) and *Carica papaya* on BWs of control treated with $CdCl_2$. Values are given as mean ± SEM each group. *: P<0.05; **: P<0.01; ***: P<0.001. Cd: Cadmium chloride toxicity; AV: Avocado; PA: Papaya.

4.2. Effect of Treatment on Spatial Learning in Morris Water Maze Test

This test was used to examine the effect of *Persea Americana* and *Carica papaya* fruit pulp juices on Cd impaired memory and learning in Cd rat's model. Figure 4.2 – 4.6 is

the representative result we reported in our study on the above mentioned fruit's effect on memory deficits in Cd rat's model

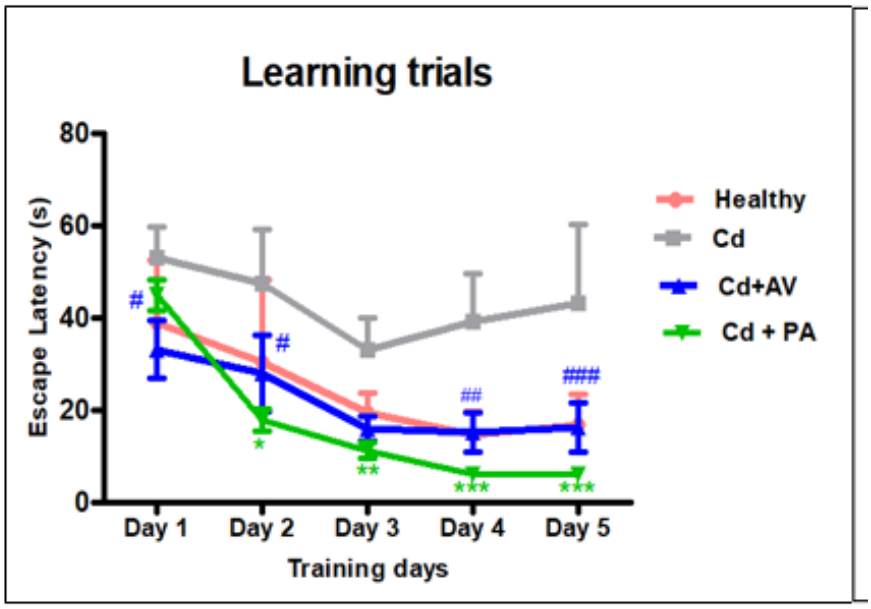

Figure 4.2 Effect of *Persea americana* and *Carica papaya* treatment against $CdCl_2$ on spatial learning and memory in Morris water maze test: The Morris water maze parameter: Escape latency for 5 days. Values are given as mean + SEM. **: P <0.01; ***: P<0.001 comparison of papaya with Cd intoxicated group . #: P<0.05; ##: P<0.01, ###: P<0.001 comparison of avocado compared with Cd intoxicated group. Cd: Cadmium chloride toxicity; AV: Avocado; PA: Papaya.

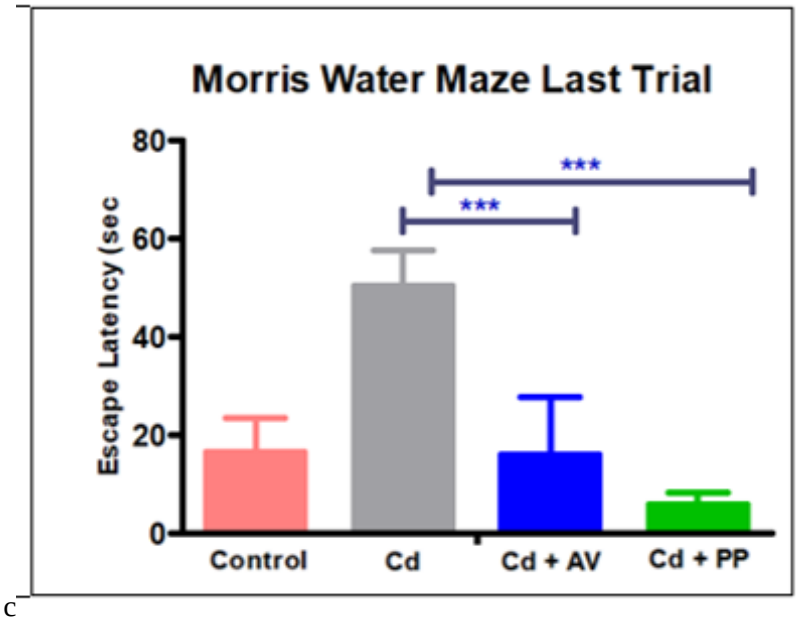

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Figure 4.3 Effect of *Persea americana* and *Carica papaya* treatment against $CdCl_2$ on spatial learning and memory in Morris water maze test: The Morris water maze parameter: Escape latency on last day. Values are given as mean + SEM. **: P <0.01; ***: P<0.001 compared with Cd intoxicated group. Cd: Cadmium chloride toxicity; AV: Avocado; PA: Papaya.

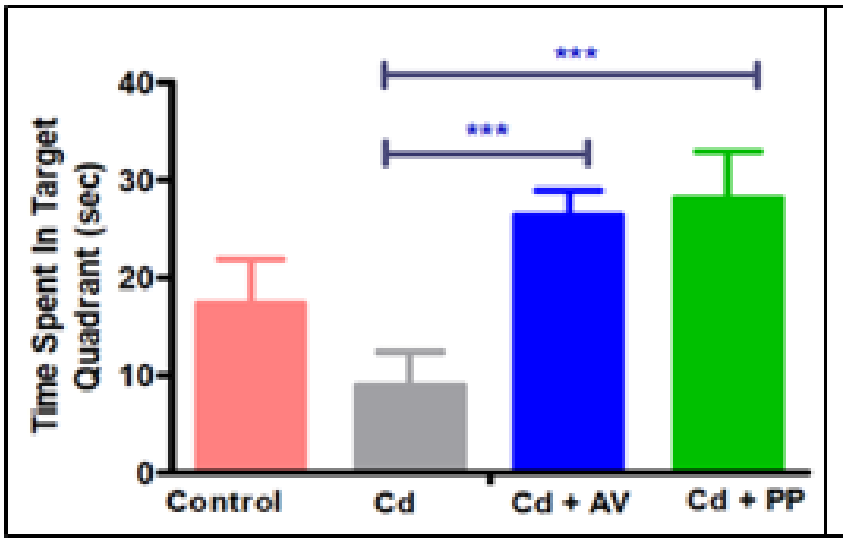

Figure 4.4 Effect of *Persea americana* and *Carica papaya* treatment against $CdCl_2$ on spatial learning and memory in MWM test. This figure shows time spent in target quadrant on probe trial. Values are given as mean + SEM. **: P <0.01; ***: P<0.001 compared with Cd intoxicated group. Cd: Cadmium chloride toxicity; AV: Avocado; PA: Papaya.

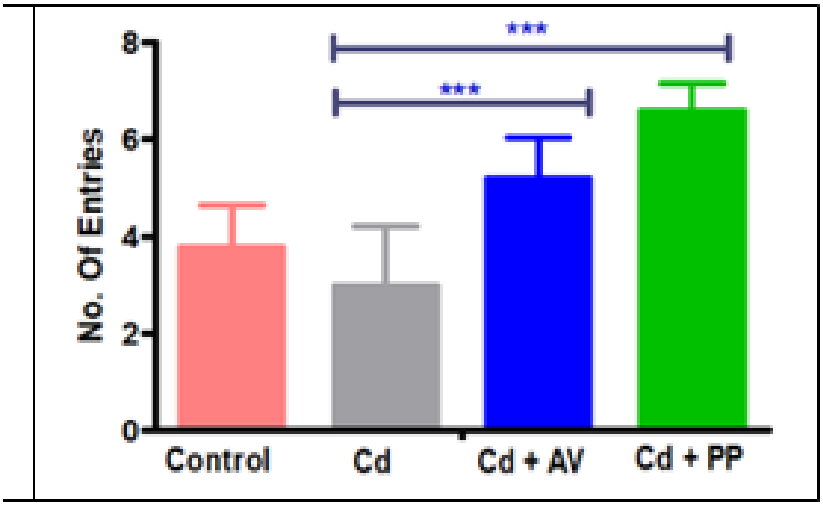

Figure 4.5 Effect of *Persea americana* and *Carica papaya* treatment against $CdCl_2$ on spatial learning and memory in Morris water maze test: The MWM probe trial parameter: No. of Entries. Values are given as mean + SEM. **: P <0.01; ***: P<0.001 compared with Cd intoxicated group. Cd: Cadmium chloride toxicity; AV: Avocado; PA: Papaya.

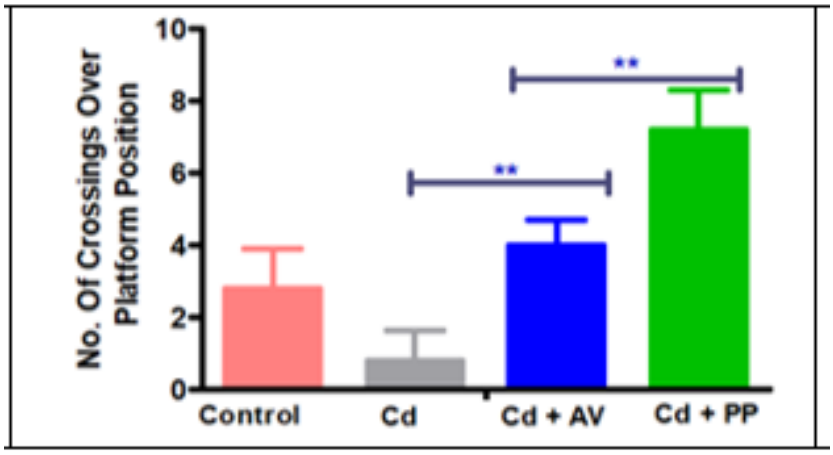

Figure 4.6 Effect of *Persea americana* and *Carica papaya* treatment against $CdCl_2$ on spatial learning and memory in Morris water maze test: The MWM probe trial parameter: No. of crossings over platform position. Values are given as mean + SEM. **: P <0.01; ***: P<0.001 compared with Cd intoxicated group. Cd: Cadmium chloride toxicity; AV: Avocado; PA: Papaya.

The results of our experiment showed significant ($p<0.01$) impairment of spatial memory in Cd model compared to the control group (Figure 4.3). However, treatment with *Persea americana* and *Carica papaya* fruit pulp juices after Cd intoxication showed a very protective effect by significantly improving memory and spatial learning in intoxicated rats. The comparison of the last day escape latency time revealed that the papaya treated animals found the platform in significantly ($p<0.001$) shorter time followed by avocado ($p<0.01$) compared to Cd intoxicated rats and control (Figure 4.3).The probe trial results showed that the *Carica papaya* treated animals spent significantly more time in target quadrant ($p<0.001$), more number of crossings over platform position ($p<0.001$) and more number of entries ($p<0.001$) followed by *Persea americana*, control and Cd intoxicated rats (Figure 4.4, 4.5 & 4.6)

4.3. Effect of Treatment on ALT, ALP, Total Bilirubin, Creatinine, and Urea

Exposure of Cd increases the ALT, ALP, total bilirubin, creatinine, and urea at the rate of 38.11%, 43.31%, 25%, 28.88%, and 51.98% respectively when compared to control.

In contrast, intoxicated rats treated with *Persea americana* decreased the liver and kidney marker enzymes such as ALT 65.17%, ALP 10.37%, total bilirubin 16.66%, creatinine 33.33% and urea 75.81% as related to intoxicated rats group.

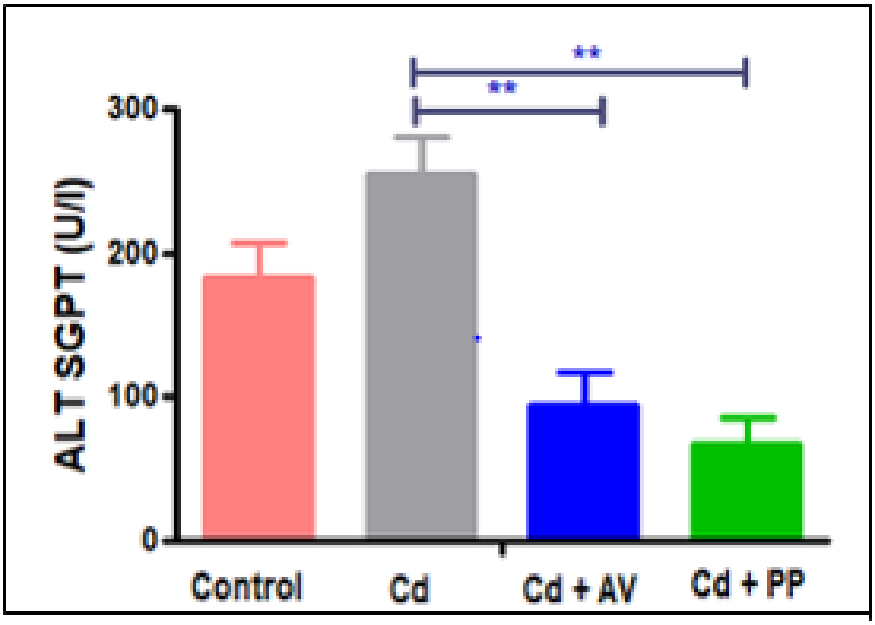

Figure 4.7 Effect of *Persea americana* and *Carica papaya* on ALT (U/l) of rats treated with $CdCl_2$. Values are given as mean ± SEM each group. *: P<0.05; Cd: Cadmium chloride toxicity; AV: Avocado; PA: Papaya; ALT: Alanine Transaminase.

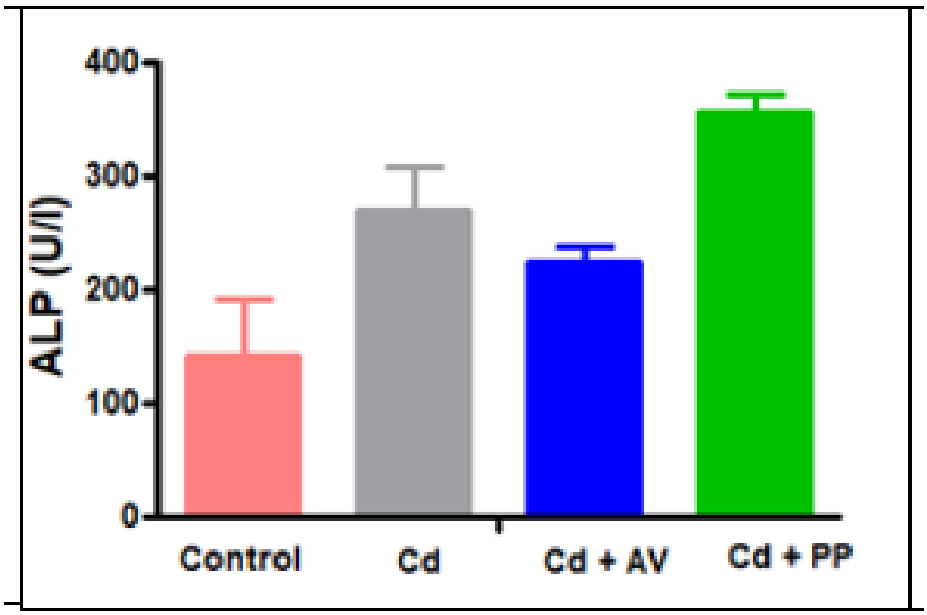

Figure 4.8 Effect of *Persea americana* and *Carica papaya* on ALP (U/l) of rats treated with $CdCl_2$. Values are given as mean ± SEM each group. *: P<0.05; Cd: Cadmium chloride toxicity; AV: Avocado; PA: Papaya; ALP: Alkaline Phosphatase.

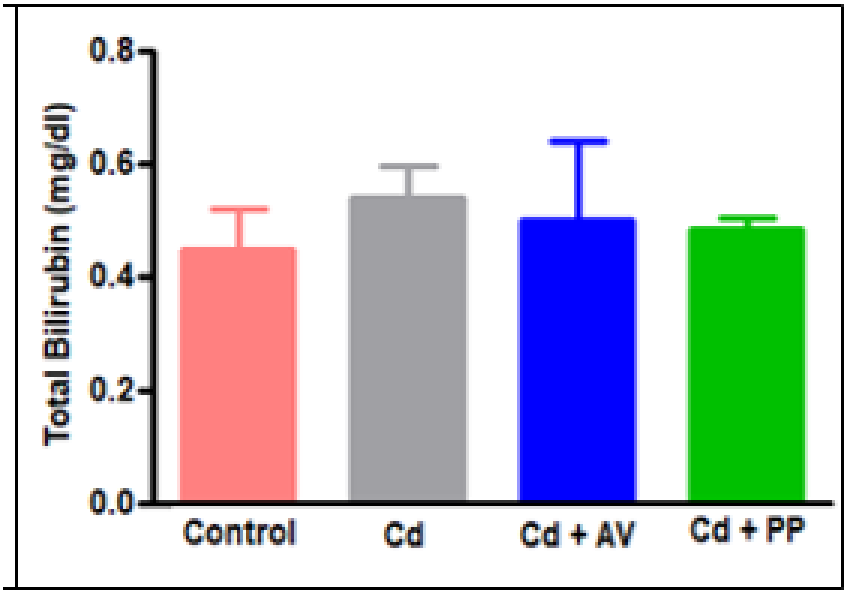

Figure 4.9 Effect of *Persea americana* and *Carica papaya* on total bilirubin (mg/dl) of rats treated with $CdCl_2$. Values are given as mean ± SEM each group. *: P<0.05; Cd: Cadmium chloride toxicity; AV: Avocado; PA: Papaya.

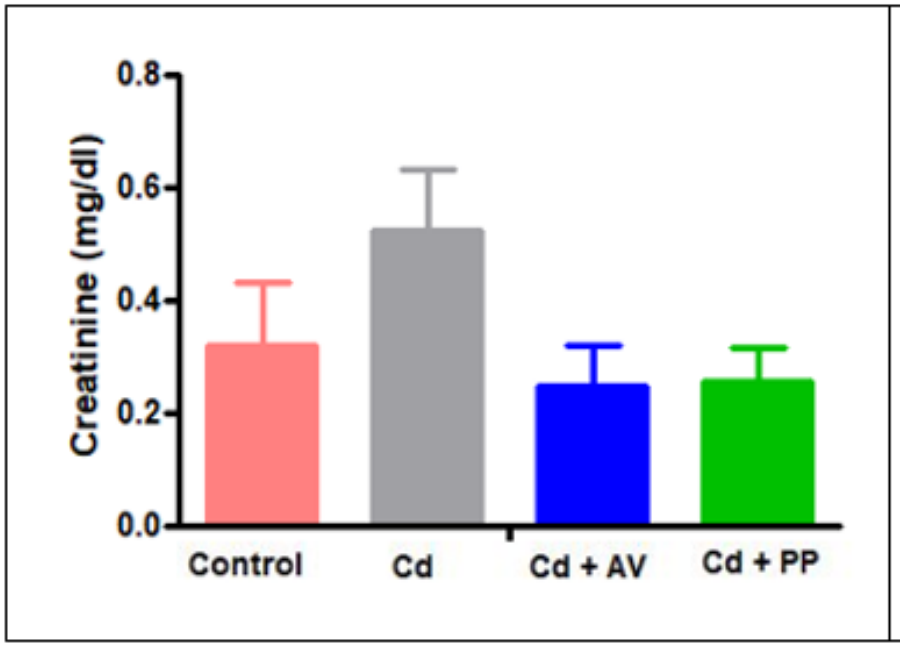

Figure 4.10 Effect of *Persea americana* and *Carica papaya* on creatinine (mg/dl) of rats treated with $CdCl_2$. Values are given as mean ± SEM each group. *: P<0.05; Cd: Cadmium chloride toxicity; AV: Avocado; PA: Papaya.

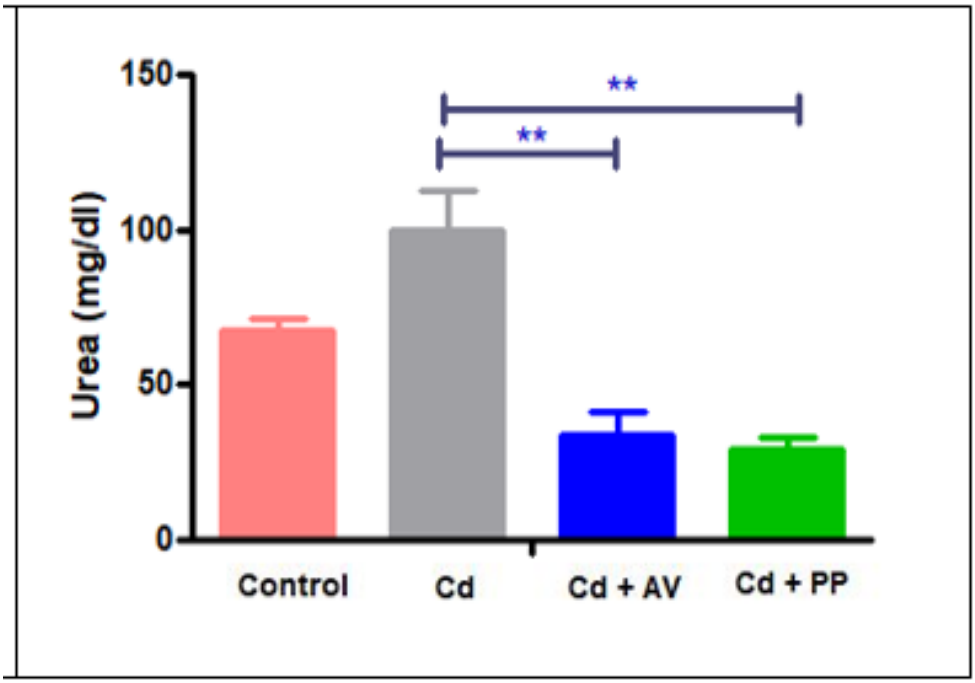

Figure 4.11 Effect of *Persea americana* and *Carica papaya* on Urea (mg/dl) of rats treated with $CdCl_2$. Values are given as mean ± SEM each group. *: P<0.05; Cd: Cadmium chloride toxicity; AV: Avocado; PA: Papaya.

Comparatively, *Carica papaya* dwindled the level of ALT (73.28%) more profoundly than *Persea americana*, however level of urea and creatinine decreased by 74.36% and 44.44% respectively when related to intoxicated rats as shown in Figure 4.7 – 4.11. An exception has seen in case of ALP level on intake of *Carica Papay*a as it has increased up to 35.76% when compared to intoxicated rats.

4.4. Effect of Treatment on Lipid Peroxidation in Liver, Kidney, and Hippocampus

Changes in MDA levels are illustrated in Figure 4.12. The level of MDA was increased significantly ($p<0.001$) in Cd intoxicated rats. After the post treatment with avocado fruit juice, the level of MDA was reduced but in the case of papaya pulp juice post-treatment, the level of MDA was lowered more significantly ($p<0.001$) in liver, kidney and hippocampus.

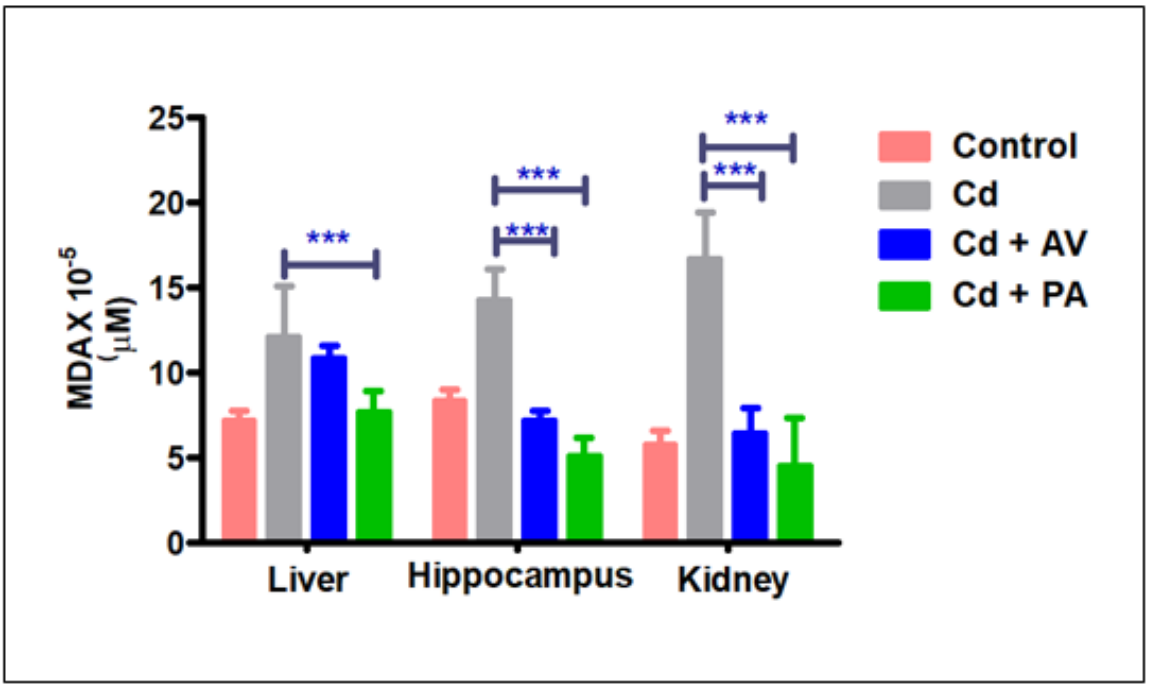

Figure 4.12 Effects of *Persea americana* and *Carica papaya* on MDA X 10^{-5} (µM) in liver, hippocampus, and kidney of rats treated with $CdCl_2$. Values are given as mean ± SEM each group. *: P<0.05; **: P<0.01; ***: P<0.001. Cd: Cadmium chloride toxicity; AV: Avocado; PA: Papaya.

The toxicity in rats decreased the MDA level by a rate of 19% in liver, 46.07% in hippocampus and 49% in kidney by *Persea americana*, while in the case of *Carica papaya*, the rate of reduction in the level of MDA was found to be 42.2% in liver, 61.68% in hippocampus and 64.1% in kidney. The *Carica papaya* treatment alleviates the level of MDA profoundly than *Persea americana* when compared to intoxicated rats.

4.5. Effect of Treatment on Catalase in Liver, Kidney, and Hippocampus

Exposure to $CdCl_2$ produced significant changes in the liver, kidney and hippocampus redox status. A very significant decrease ($p<0.001$) in CAT levels activities was recorded in intoxicated groups in liver and kidney by 78.49% and 78.57% respectively while in hippocampus significant ($p<0.01$) decrease was 78.94% compared to controls (Figure 4.13).

Administration of *Persea americana* in diet after Cd exposure showed an amelioration in CAT by significantly ($p<0.001$) increasing their values at the rate of 75.60% in liver, 86.87% in kidney and 88.32% in hippocampus while in case of *Carica papaya* intake increased the CAT values by 75.30%, 87.93% and 89.54% in liver, kidney, and hippocampus respectively.

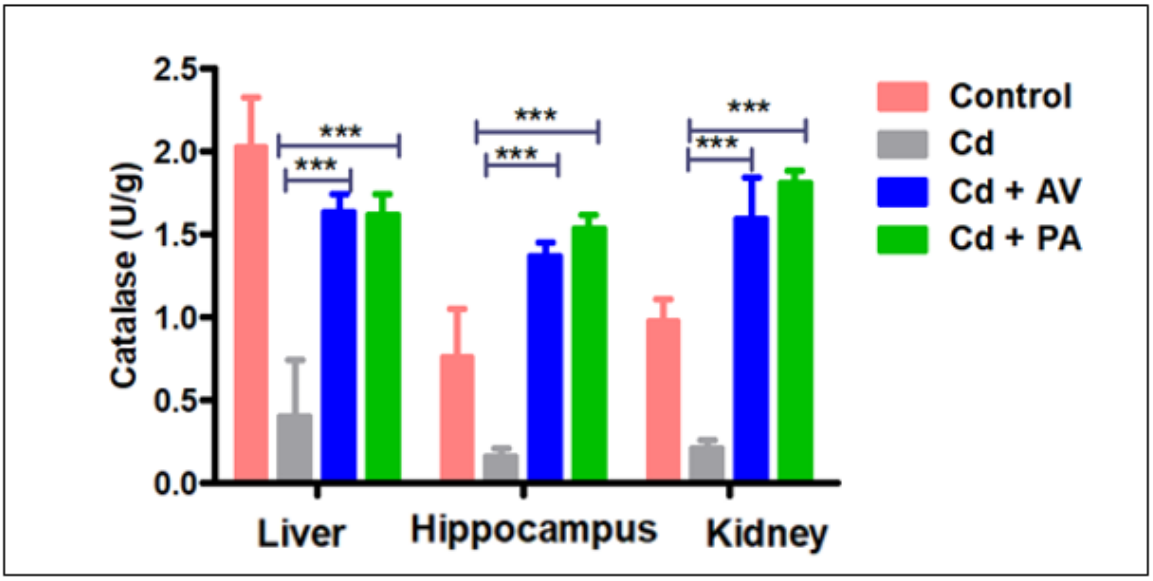

Figure 4.13 Effects of *Persea americana* and *Carica papaya* on Catalase (U/g) in liver, hippocampus, and kidney of rats treated with $CdCl_2$. Values are given as mean ± SEM each group. *: P<0.05; **: P<0.01; ***: P<0.001. Cd: Cadmium chloride toxicity; AV: Avocado; PA: Papaya.

The *Carica papaya* showed more efficient results than *Persea americana* in terms of improving and enhancing antioxidant status in intoxicated rats.

4.6. Effect of Treatment on Gene Expression of SOD, CAT & GPx

The SOD, CAT and GPX expression was downregulated in cadmium chloride treated group of rats as compared to control. The expression of SOD was significantly upregulated after treatment of cadmium intoxicated rats with papaya juice in hippocampus ($p<0.01$), kidney ($p<0.01$) and liver tissues ($p<0.001$). The expression of CAT and GPX was also found to be upregulated more actively in papaya as compared to avocado.

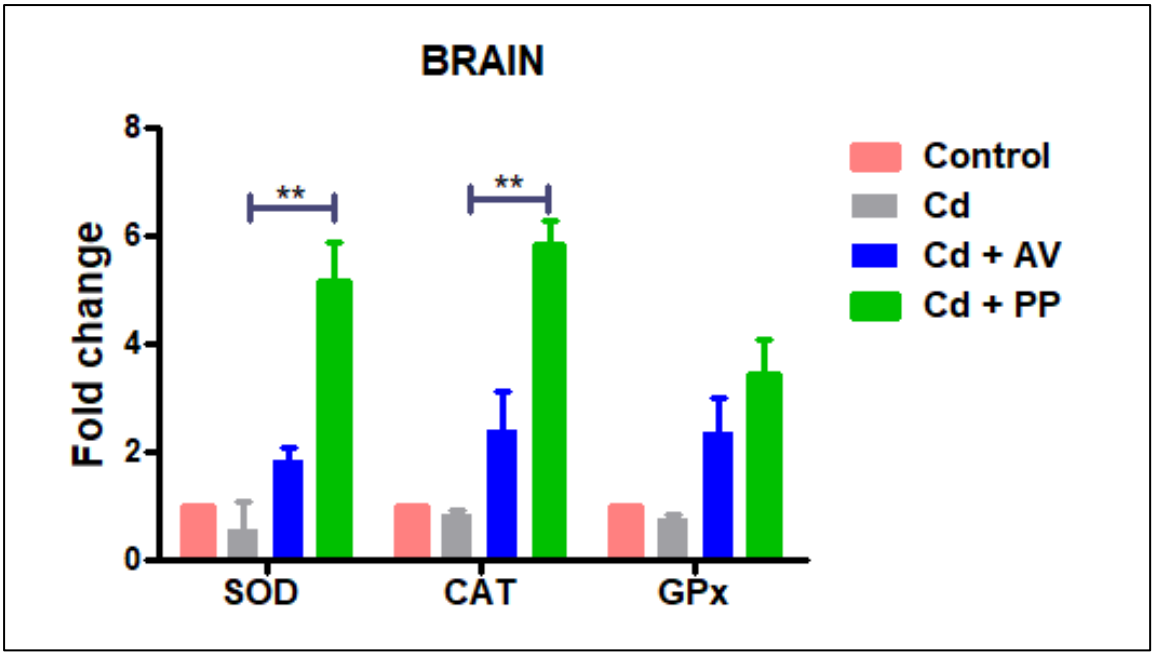

Figure 4.14. Comparison of SOD, CAT and GPx expression in hippocampus in cadmium positive group and post treatment avocado and papaya group. Values are given as mean ± SEM each group. *: P<0.05; **: P<0.01; ***: P<0.001. Cd: Cadmium chloride toxicity; AV: Avocado; PA: Papaya

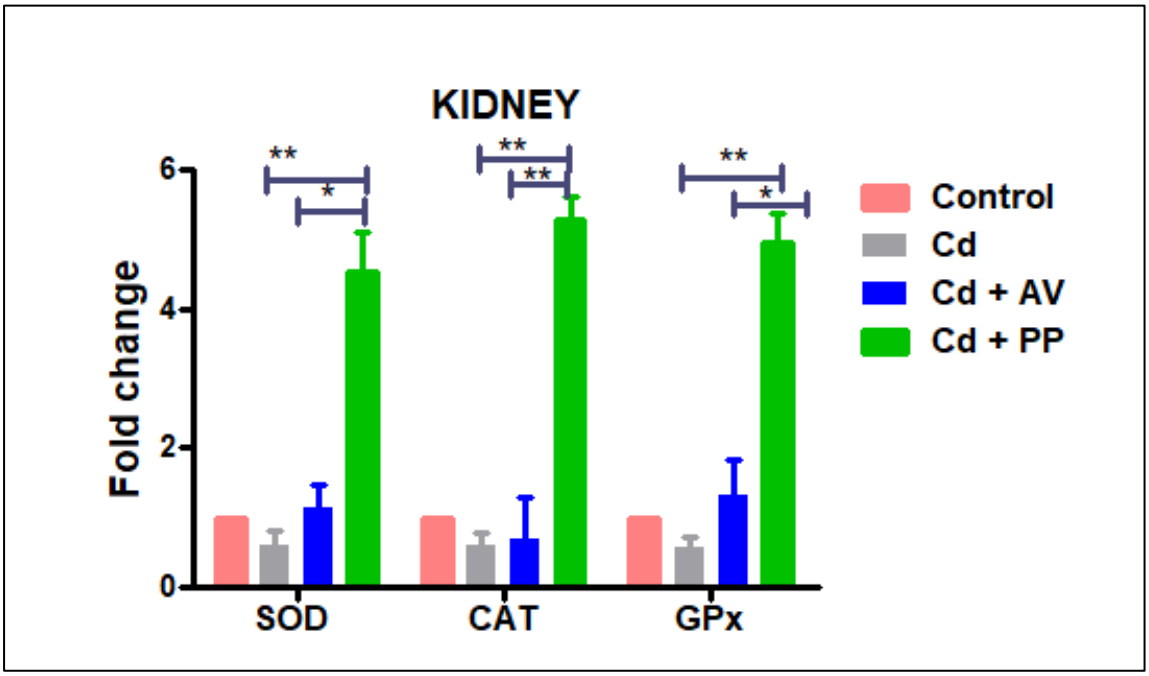

Figure 4.15 Comparison of SOD, CAT and GPx expression in kidney in cadmium positive group and post treatment avocado and papaya group. Values are given as mean ± SEM each group. *: P<0.05; **: P<0.01; ***: P<0.001. Cd: Cadmium chloride toxicity; AV: Avocado; PA: Papaya

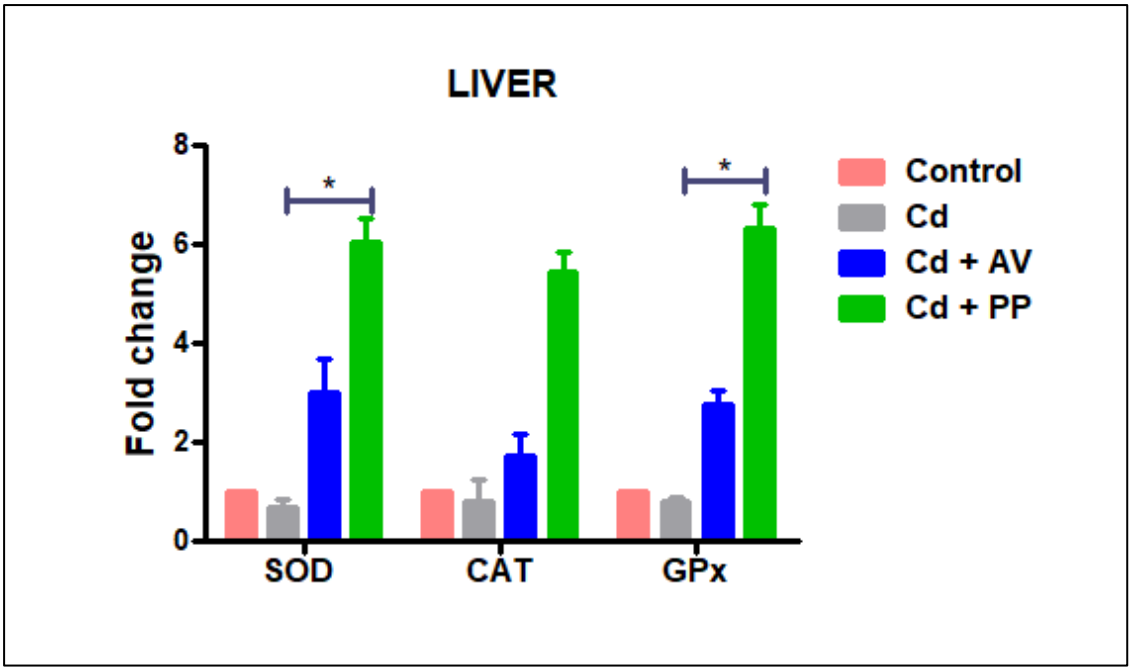

Figure 4.16 Comparison of SOD, CAT and GPx expression in hippocampus in cadmium positive group and post treatment avocado and papaya group. Values are given as mean ± SEM each group. *: P<0.05; **: P<0.01; ***: P<0.001. Cd: Cadmium chloride toxicity; AV: Avocado; PA: Papaya

CHAPTER 05

DISCUSSION

Cadmium is foremost present in cigarettes, food and drinking water. It is a noxious metal that causes oxidative stress in most of the tissues such as liver, kidney, and hippocampus (Oguzturk *et al*., 2012). It retains in the tissues for a long time and causes serious pathological ramification (Bagchi *et al*., 1997). The mechanism behind the damage caused by cadmium is to create reactive oxygen species and amend the normal functioning of the cell by disrupting genetic information and mitochondrial activity (Patrick *et al*., 2002). The present study has evidently explained the toxicity of Cd by inducing ROS in rat's liver, kidney, and hippocampus as testified by an increase in lipid peroxidation after 7 weeks of Cd exposure. The related finding has been observed with the reports on enhanced lipid peroxidation with Cd exposure (Fatma M. El-Demerdash *et al*., 2004 ; Y. Shen *et al*., 1995). The current work was mainly focused on the potential protective effects of *Persea americana* and *Carica papaya* and their comparative analysis against Cd-induced neurotoxicity, nephrotoxicity, and hepatotoxicity.

The mean body weight of cadmium decreases in the present research as compared to control as shown in figure 1. This finding reconciles with the previous report (F. M. El-Demerdash *et al*., 2004) suggested that the risk of diabetes mellitus increases with the cadmium toxicity and it might be the reason of weight loss. (Kaltreider *et al*., 2001) reported in findings that the exposure of low levels of metal can impair the glucocorticoid system that majorly involves in the metabolism of carbohydrates, lipids, and proteins. Thus the glucocorticoid system has a link with the gain and loss of weight.

Being a neurotoxin, cadmium alters the memory and learning in rats observed in MWM as shown in figure 2. Previous studies have explained that its exposure debilitates memory and learning functions (Haider *et al*., 2015). In the underlying study, administration of cadmium impaired the memory significantly by taking more time for escape latency after the training session in the MWM test as related to control. This might be due to the reduction in acetylcholinesterase activity in hippocampus that is related to the memory impairment in rats administered with Cd.

The elevated serum level of liver ALT and ALP with $CdCl_2$ toxicity as shown in figure 3 (A& B), are suggestive of loss of functional integrity of cell membrane and cellular leakage in the liver (Williamson *et al*., 1996). The increasing biliary pressure causes the increased synthesis of ALP in serum (Burtis *et al*., 1998). The similar finding was reported by (Rana *et al*., 1996) , who found variations in the level of hepatic transaminases on exposure with Cd in rats. Similar findings have been reported in which ALT and ALP level increases when rats were treated with $CdCl_2$ (F. M. El-Demerdash *et al*., 2001).

The significant increase in the level of creatinine and urea after the exposure of cadmium as compared to the control group was shown in figure 3 (D & E). This indicates the deterioration in the kidney function. The similar increase in the level of creatinine and urea with Cd has been observed in previous reports (Mohammed *et al*., 2014; Novelli *et al*., 1998). Creatinine is the most preferable renal marker and it increases on the damage of major portion of the kidney while urea is the foremost acute renal market and it exceeds on renal injury (Borges *et al*., 2005). The variation in levels of both creatinine and urea indicates a seriously damaging effect of $CdCl_2$ in the kidney.

The cadmium-induced neurotoxicity, nephrotoxicity, and hepatotoxicity was evident from the critical event of the impaired antioxidant defense system and elevated lipid peroxidation as shown in figure 4 & 5. In the present study, lipid peroxidation was determined with the measurement of MDA. The enhanced level of MDA was observed in liver, kidney, and hippocampus in rats treated with $CdCl_2$ when compared to control (figure 4). These findings were reinforced by (Manca *et al*., 1991), reporting that lipid peroxidation is the initial and responsive consequence of cadmium toxicity (Kawamoto *et al*., 2007) revealed that cadmium causing lipid peroxidation has a pernicious effect on membrane-dependent functions.

The present analysis has shown a significant decrease in the level of antioxidant enzyme CAT. Catalase is the hemeprotein that catalyzes the hydrogen peroxide and hydroxyl radical and reduces them to water. The decrease in the level of CAT in rats treated with cadmium might be due to the overproduction of ROS by cadmium toxicity. The same finding has been reported in previous research in which level of CAT decreased in hepatic tissue (Milton Prabu *et al*., 2012; Renugadevi *et al*., 2010) while (Erejuwa *et al*., 2011) observed amelioration in CAT level in renal tissues. In the case of brain tissues, decrease in CAT level has been observed in microvessels leading to dysfunctioning of the blood-brain barrier (Shukla *et al*., 1996), in other cases decline has been observed in catalase level in the corpus striatum and cerebral cortex (Pal *et al*., 1993). Similar findings with hippocampus with cadmium had also been observed in previous researches (Kanter *et al*., 2016).

The level of SOD, CAT and GPx was found to be downregulated in cadmium treated group in our study. The activity and expression of antioxidant enzymes was reduced by cadmium. (Almeer *et al*., 2019) observed the decrease in the level of antioxidant enzymes in kidney tissues when rats were injected with cadmium chloride. Our results

of this research were supported by previous research which revealed that cadmium caused renal toxicity and hepatotoxicity by improving ROS growth, GSH consumption and antioxidant-mediated defense system inhibition (Renugadevi *et al*., 2010; R. Shen *et al*., 2017). The SOD catalyzes the conversion to H_2O_2 of superoxide anion. SOD catalyzes a dismutation response to the conversion of the superoxide anion to H_2O_2. Cd may interfere with other SOD cofactors and thus inactivate the enzyme besides interacting with critical SOD protein groups. CAT is an important antioxidant enzyme with a prosthetic group based on hem as an active site. Cd is known to reduce assimilation of iron and impair heme biosynthesis. The activity of GPx was inhibited in the current research. When Cd is accumulated in the brain, it can interact with GPx's active core (Se-Cys) and lead to structural modifications that inactivate GPx that correlates with the previous reports (Abdel Moneim *et al*., 2014; Zhang *et al*., 2006).

In the present study, treatment with *Carica papaya* fruit pulp after inducing cadmium toxicity significantly restored the memory, antioxidant level of all tissues (liver, kidney & hippocampus), biochemical parameters (ALT, creatinine urea and total bilirubin) and lipid peroxidation. Similar reports were found on hepatoprotective properties of *Carica papaya* against carbon tetrachloride toxicity (Sadeque *et al*., 2010). Similar findings have been reported by (Pandit *et al*., 2013; Rajkapoor *et al*., 2002; Sadeque *et al*., 2010). In his research, an exception has observed in case of level of ALP. Treatment with *Carica papaya* enhanced the level of ALP. This might be due to the presence of ALP in abundant amount in overripe Local pawpaw that was used in the present research (Agoreyo *et al*., 2014). Moreover increased bone activities could be the reason for the escalated level of ALP (Oduola *et al*., 2007). Unripe *Carica papaya* seed extract has nephroprotective properties against carbon tetrachloride toxicity as it scavenges the ROS by decreasing MDA and increasing CAT (Adeneye *et al*., 2009). Previous studies

have reported that *Carica papaya* contains phenolic compounds (Patthamakanokporn *et al*., 2008). Phenolic compounds exhibit anti-lipid peroxidation activity that has a high tendency to chelate metals. Phenolics bind with the iron and copper with its hydroxyl and carboxyl groups and inactive them by chelation. It results in the suppression of superoxide-mediated Fenton reaction (Michalak *et al*., 2006). It also contains ascorbic acid that particularly reduces ferric ions and inhibits lipid peroxidation. Carotenoids are also present in it that reduces the oxygen by donating hydrogen. Hence, reduces the formation of ROS (Rekha *et al*., 2012). In previous reports, it has been reported that *Carica papaya* leaf extract proved to be neuroprotective against fluoride causing neurotoxicity. It has successfully rectified memory and learning in rats (Banala *et al*., 2018).

In the present study, treatment with *Persea americana* fruit pulp significantly restored memory & learning, level of ALT, ALP, Total bilirubin, urea, and creatinine. This indicates the neuroprotective, hepatoprotective and nephroprotective effect of *Persea americana* against cadmium toxicity. The antioxidant activities of *Persea americana* seeds and leaves have been reported in previous work (Oboh *et al*., 2016). Avocado oil has been observed to improve mitochondrial function and oxidative stress in the brain of diabetic rats (Ortiz-Avila *et al*., 2015). Aqueous extract of *Persea americana* possesses significant hepatoprotective role against CCl_4 induced toxicity (Brai *et al*., 2014). The scavenging activity and antioxidant activity of *Persea americana* are due to the presence of flavonoids and phenolic compounds (Arukwe *et al*., 2012; Nabavi *et al*., 2013). Ethanolic extract of *Persea americana* seeds have been reported to be effective against renal toxicity caused by potassium aluminium sulphate and hepatoprotective against gentamicin-induced nephrotoxicity (Nabayra *et al*., 2009). *Persea americana* has a role in the restoration of damaged renal tubules necrosis. This

may be due to the presence of flavonoids in ethanolic extract of *Persea americana* leaves (Fakultas Kedokteran Hewan *et al*., 2014).

The expression of antioxidant enzymes was found to be upregulated after post treatment of Cd intoxicated group with avocado and papaya juice. The level of SOD, CAT and GPX was found to be upregulated in papaya juice more actively as compared to avocado. It was found that ascorbic acid, caffeic acid, glucosinolates, kaempferol, and papain are present in *Carica papaya* that has anticancer, antioxidant and radical scavenging property. The similar report in the reduction of SOD has been observed in previous report with *P. ginseng and S. platensis* (Karadeniz *et al*., 2009). The effect of *Carica papaya* and *Persea americana* is attributable both to its being a SOD and GPx stimulator. Similar findings were found with *Spirulina platensis* against Cd induced toxicity and dysfunctions of many organs (Preet).

After comparatively analyzing the protective role of *Carica papaya* and *Persea americana* against cadmium-induced neurotoxicity, nephrotoxicity, and hepatotoxicity, it has been found that *Carica papaya* activity has high antioxidant activity when compared to *Persea americana.*

CONCLUSION

It is evident from this study that *Carica papaya* maintains the cellular redox balance more actively than *Persea americana* by lowering the level of reactive oxygen species. It was determined that *Persea americana* and *Carica papaya* both are able to lowering the level of MDA when compared to Cd rat model but the results were found to be more significant in case of *Carica papaya*. On the basis of MWM test, it seems that *Carica papaya* improves the spatial learning and memory more profoundly than *Persea americana* against Cd induced memory deficit rats. *Carica papaya* and *Persea americana* upregulates the level of antioxidant enzymes SOD, CAT & GPx but the role of *Carica papaya* was significantly more as compared to *Persea americana*. Hence proved that *Carica papaya* exhibits strong antioxidant activity as compared to *Persea americana* against Cd induced toxicity in liver, kidney and hippocampus. So, we can conclude that diet rich in *Carica papaya* and *Persea americana* could be an excellent antioxidant source. Their intake may therefore provide an alternative strategy to prevent oxidative stress damage caused by toxin such as Cd.

FUTURE PROSPECTS

1. Further research is required to elucidate dose dependent antioxidant activity of *Carica papaya and Persea americana* against cadmium toxicity in vivo
2. The leaves, peel & seeds extract of *Carica papaya* and *Persea americana* can also be investigated against cadmium toxicity
3. The antioxidant effects of other fruits and vegetables can also be compared against cadmium toxicity

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