DETERMINATION OF ANTIOXIDANT AND METAL CHELATING PROPERTIES OF TEA (*Camelia sinensis*) IN AMELIORATING CADMIUM INDUCED TOXICITY IN MALE WISTAR RATS

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A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Degree of Master of Science in Biochemistry of Egerton University

EGERTON UNIVERSITY

JULY , 2020

DECLARATION AND RECOMMENDATION

Declaration

I declare that this thesis is my original work and has not been submitted wholly or in part in this form or any form for a degree in this or any other university.

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Recommendation

This thesis has been submitted with our approval as University supervisors.

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DEDICATION

I would like to dedicate this thesis to my parents and family who made this MSc program possible.

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ABSTRACT

Cadmium (Cd) is a ubiquitous environmental and industrial pollutantwhose exact toxicity mechanisms still remain elusive. However, cumulative data has implicated bioaccumulation as well as free radical generation in the biochemical and molecular mechanisms of cadmium induced toxicity. Chelation therapy with calcium disodium ethylenediamine tetra acetic acid ($CaNa_2EDTA$), British Anti Lewisite (BAL), meso 2, 3-dimercaptosuccinic acid (DMSA) is so far among the best known treatment against heavy metal poisoning. Nevertheless, this treatment is compromised by grave side-effects such as depletion of essential metals, inability to pass through the cellular membranes and redistribution of the metals to the body organs etc.This study evaluated the modulatory effects of Kenyan black and green tea extracts in comparison with Na_2EDTA on experimentally induced cadmium toxicity in the brain, liver, kidney, testes and bones of male wistar albino rats. This is because tea is non-toxic and is endowed with metal chelating and antioxidant properties as well as its ability to cross the blood brain barrier (BBB). Subcutaneous administration of cadmium chloride induced renal, hepatic, neuronal, testicular and bone damage which was evident from the significantly ($P<0.05$) increased levels of serum AST, ALT, ALP as well as decreased levels of total proteins and albumin in addition to a significant ($p < 0.05$) decrease in ZHX1 in brain and liver tissue homogenates. Significantly increased levels of lipid peroxidation markers Thiobarbituric Acid Reactive Substances with significant ($p < 0.05$) increase in reduced glutathione as well as increased levels of cadmium in the liver, kidney, testes and bones were also observed in cadmium-treated rats. Co-administration of aqueous black or green tea extracts along with Cd resulted in a reversal of Cd-induced biochemical changes in liver and brain accompanied by a significant decrease in lipid peroxidation and an increase in the level of hepatic, neuronal, testicular and renal antioxidant defense system. There was also a significant ($p<0.05$) reduction in cadmium chloride levels in the liver, kidney, testes and bone tissues. The histopathological studies in the brain, liver, kidney and testes of rats also showed that the aqueous extracts of black and green tea significantly reduced toxicity of Cd and preserved the normal histological architecture of the tissues examined. This study attributes the cytoprotective potential of tea in Cd toxicity to its antioxidant and metal chelating properties. Furthermore, data from this study provides novel insights on mechanisms of cadmium toxicity and may possibly give a lead to tea product diversification in new regimens or pharmacological interventions against heavy metal toxicity.

TABLE OF CONTENTS

DECLARATION AND RECOMMENDATION ..... **ii**
COPYRIGHT ..... **iii**
DEDICATION ..... **iv**
ACKNOWLEDGEMENTS ..... **v**
ABSTRACT ..... **vi**
TABLE OF CONTENTS ..... **vii**
LIST OF TABLES ..... **xi**
LIST OF FIGURES ..... **xii**
LIST OF ABBREVIATIONS AND ACRONYMS ..... **xiv**
CHAPTER ONE **1**
INTRODUCTION **1**
1.1 Background Information **1**
1.2 Statement of the Problem **3**
1.3 Objectives **3**
1.3.1 General objective 3
1.3.2 Specific objectives 3
1.4 Hypotheses **4**
1.5 Justification **4**
CHAPTER TWO ***5***
LITERATURE REVIEW ***5***
2.1 Heavy Metals **5**
2.2 Cadmium Toxicity **5**
2.3 Absorption of Cadmium **6**
2.4 Cadmium and Oxidative Stress **7**
2.5 Effects of Cadmium on ZHX1 ..... **7**
2.6 Effect of Cd on GSH levels **8**
2.7 Effect of Cd on Lipids **9**
2.8 Role of Cd on tissue histology and histopathology **10**
2.9 Interactions between Cd and essential metals **10**
2.10 Chelation Therapy **10**
2.21 Types of tea and their distribution in Kenya **11**
2.22 Isolation and characterization of tea polyphenols **11**

CHAPTER THREE ***14***
MATERIALS AND METHODS ***14***
3.1 Tea Samples and Processing **14**
3.2 Sample Treatment for Polyphenol and Catechin Analysis **14**
3.2.1 Determination of Catechins in Tea Infusions 15
3.2.2 Determination of Total Polyphenols in the Tea Infusions 15
3.2.3 Analysis of Total Theaflavins Content in the Tea Infusions 16
3.2.4 Determination of Total Thearubigins in Tea Infusions 17
3.2.5 Free Radical Scavenging Activity of Tea Samples 17
3.2.6 Determination of Heavy Metals in Tea Infusions 18
3.3 Experimental Rats **19**
3.4 Experimental Design **19**
3.4.1 Determination of Packed Cell Volume (PCV) and Body Weight 20
3.4.2 Tissue Homogenization 20
3.4.3 Determination of Reduced Glutathione 20
3.4.4 Zinc Fingers and Homeoboxes Protein 1 (ZHX1) 21
3.4.5 Thiobarburic Acid Reactive Substances (TBARS) Assay 21
3.4.6 Determination of Alanine Aminotransferase (ALT) 22
3.4.7 Determination of Aspartate Aminotransferase (AST) 22
3.4.8 Determination of Alkaline Phosphatase(ALP) 22
3.4.9 Determination of Total Protein Levels 23
3.4.10 Determination of Serum Albumin Levels 23
3.4.11 Determination of Cadmium Chloride in Tissues 23
3.5 Evaluation of Brain, Liver, Testes and Kidney's Pathological Changes **24**
3.5.1 Hematoxylin and Eosin (H and E) Staining 24
3.5.2 Brain Immunohistochemistry 24
3.6 Data Analysis **24**
CHAPTER FOUR ***25***
RESULTS ***25***
4.1 Analysis of Tea **25**
4.2 Effects of Tea and EDTA on ZHX1. TBARS and GSH levels in the Brain of Rats **28**
4.2.1 Signs and Symptoms 28
4.2.2 Percent Packed Cell Volume (PCV) Levels. 28
4.2.3 Body Weight of Rats 29

4.2.4 Brain Reduced Glutathione Levels 29
4.2.5 Brain Zinc Fingers and Homeoboxes Protein I Levels 30
4.2.6 Brain Thiobarburic Acid Assays Levels 31
4.3 Effects of tea (Black Tea and Green Tea) aqueous Extracts Compared with EDTA Consumption on the liver healthy wistar rats **32**
4.3.1 Liver Reduced Glutathione (GSH) Levels 32
4.3.2 Liver Zinc Fingers and Homeoboxes Protein I (ZHX1) Levels 32
4.3.3 Liver Thiobarburic Acid Assays (TBARS) Levels 33
4.4 Liver Function Tests (LFTs) **34**
4.5 Protective Effects of Aqueous Black Tea and Green Tea Extracts Compared with EDTA during Cadmium Induced Toxicity in the Brain of Rats **37**
4.5.1 Clinical Signs and Symptoms 37
4.5.2 Percent (%) Packed cell Volume levels 37
4.5.3 Body Weight Levels 38
4.6 Effects of Aqueous Black Tea and Green Tea Extracts Compared with EDTA during Cadmium Induced Toxicity in the Brain of Rats **39**
4.7 Effects of Aqueous Black Tea and Green Tea Extracts Compared with EDTA during Cadmium Induced Toxicity in the Liver of Rats **41**
4.8 Liver Function Tests **44**
4.9 $CdCl_2$ levels in Various Body Tissues **49**
4.10 Histopathology and Immunohistochemistry **53**
4.11 Discussion **61**
4.11.1 Tea flavonoids of Kenyan tea cultivar 61
4.11.2 Effects of tea on the liver function and ZHX1 rats 64
4.11.3 Cadmium chloride mediated effects on different tissues of rats 65
4.11.4 Protective effects of aqueous extracts of black and green tea on Cd induced toxicity in the liver and kidney of rats 71
4.11.4 Protective effects of aqueous extracts black and green tea on cadmium induced brain toxicity in rats 75
4.11.5 Protective effects of aqueous extracts black and green tea on cadmium induced testicular toxicity in rats 78

CHAPTER FIVE ***80***
CONCLUSIONS AND RECOMMENDATION ***80***
5.1 Conclusions **80**
5.2 Recommendations **81**
REFERENCES **82**
APPENDICES **108**
Appendix I: Total GSH calibration curve -1 ..... **108**
Appendix II: Total GSH calibration curve -2 ..... **109**
Appendix III: Total GSH calibration curve -3 ..... **110**
Appendix IV: Total GSH calibration curve -4 ..... **111**
Appendix V: Brain TBARS calibration curve **112**
Appendix VI: Liver TBARS calibration curve **113**
Appendix VII: Institutional Animal Use and Care Approval Letter **114**
Appendix VIII: Journal PublicationI **115**
Appendix IX: Journal Publication II **116**

LIST OF TABLES

Table 1: A representation of the study design 20
Table 2: Individual catechin (%) levels of black and green tea analyzed.....27
Table 3: Percent total polyphenols (TP), antioxidant capacity (AA), theaflavins and thearubigins of black and green tea analyzed 27
Table 4: Levels of Cadmium and trace elements in tea.....27
Table 5: Percent (%) PCV changes in rats supplied with black tea, green tea or EDTA compared with rats that were supplied with water only. 28

LIST OF FIGURES

Figure 1: Mechanisms of action of tea polyphenols during heavy metal toxicity 8
Figure 2: A schematic representation of the study design 19
Figure 3: A chromatogram of Kenyan black tea variety TRFK 6/8 24
Figure 4: A chromatogram of Kenyan green tea variety TRFK 6/8 25
Figure 5: Changes in body weight of rats supplied with EDTA, black tea, green tea or water only 28
Figure 6: Effect of the chelating antioxidants on brain GSH levels in rats. 29
Figure 7: Effect of the chelating antioxidants on brain ZHX1 levels in rats. 30
Figure 8: Effect of the chelating antioxidants on brain TBARS levels in rats. 31
Figure 9: Effect of the chelating antioxidants on liver GSH levels in rats. 32
Figure 10: Effect of the chelating antioxidants on liver ZHX1 levels in rats. 32
Figure 11: Effect of the chelating antioxidants on liver TBARS levels in rats. 33
Figure 12: Effect of the chelating antioxidants on serum ALT levels in rats. 34
Figure 13: Effect of the chelating antioxidants on serum AST levels in rats. 35
Figure 14: Effect of the chelating antioxidants on serum ALP levels in rats. 35
Figure 15: Effect of the chelating antioxidants on total protein levels in rats. 36
Figure 16: Effect of the chelating antioxidants on serum albumin levels in rats. 37
Figure 17: Percent PCV changes in rats supplied with $CdCl_2$ along with black tea, green tea or EDTA compared with rats that were supplied with water only. 38
Figure 18: Changes in body weight of rats (Mean±SEM) treated with $CdCl_2$ along with EDTA, black tea, green tea or water only 39
Figure 19: Effect of $CdCl_2$ exposure with or without chelating antioxidants intervention on brain GSH levels in rats. 40
Figure 20: Effect of $CdCl_2$ exposure with or without chelating antioxidants intervention on total brain ZHX1 levels in rats. 41
Figure 21: Effect of $CdCl_2$ exposure with or without chelating antioxidants intervention on brain MDA levels in rats. 42
Figure 22: Effect of $CdCl_2$ exposure with or without chelating antioxidants intervention on liver GSH levels in rats. 43
Figure 23: Effect of $CdCl_2$ exposure with or without chelating antioxidants intervention on total liver ZHX1 levels in rats. 44
Figure 24: Effect of $CdCl_2$ exposure with or without chelating antioxidants intervention on liver MDA levels in rats. 45

Figure 25: Effect of $CdCl_2$ exposure with or without chelating antioxidants intervention on liver ALT levels in rats. 46
Figure 26: Effect of $CdCl_2$ exposure with or without chelating antioxidants intervention on liver AST levels in rats. 47
Figure 27: Effect of $CdCl_2$ exposure with or without chelating antioxidants intervention on liver ALP levels in rats. 48
Figure 28: Effect of $CdCl_2$ exposure with or without chelating antioxidants intervention on protein levels in rats. 49
Figure 29: Effect of $CdCl_2$ exposure with or without chelating antioxidants intervention on albumin levels in rats. 50
Figure 30: Effects of tea and EDTA on Levels of $CdCl_2$ in the kidney of rats. 51
Figure 31: Effects of tea and EDTA on Levels of $CdCl_2$ in the liver of rats: 52
Figure 32: Effects of tea and EDTA on Levels of $CdCl_2$ in the testes of rats. 53
Figure 33: Effects of tea and EDTA on Levels of $CdCl_2$ in the long bone of rats. 54
Figure 34: Representative photomicrograph showing histopathological changes in the rat brain. 55
Figure 35: Representative photomicrographs showing the effects of Cd, BTE, GTE and EDTA on the immunohistochemistry of the brain. 56
Figure 36: Histological sections of adult albino rats testes sections 56
Figure 37: A photomicrograph of Albino rats liver 59
Figure 38: Cross sections pf the kidneys cortex region 60

LIST OF ABBREVIATIONS AND ACRONYMS

CaNa2EDTA	Calcium disodium ethylenediamine tetra acetic acid
Cd	Cadmium
BAL	British antilewisite
DMPS	Sodium 2,3 dimercaptopropane 1 sulfonate
DMSA	meso 2, 3 dimecaptosuccinic acid
GSH	Reduced glutathione
AST	Aspartate aminotransfarase
ALT	Alanine aminotransfarase
ZHX1	Zinc fingers and homeoboxes protein 1
WHO	World health organization
NLS	Nuclear localization signal
DBD	DNA binding domain
BBB	Blood brain barrier
RPM	Revolutions per minute
RT	Room temperature
DNPH	2,4-Dinitrophenylhydrazine

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Heavy metal induced toxicity is becoming a concern due to its prevalence (Leelavinothan *et al.,* 2011). This arises from anthropogenic activities such as manufacturing activities, vehicle exhaust fumes, heavy-duty electric power engines, burning of waste materials and use of pesticides in farming (Flora *et al*., 2008; Leelavinothan *et al.,* 2011). Entry of heavy metals into the human biological system leads to overt and insidious health problems as a result of their long biological half-life (Djukić-Ćosić *et al.,* 2008). This causes metal intoxication manifested as neurotoxicity, genotoxicity or carcinogenicity (Flora *et al.,* 2008). Humans, plants and animals become incidentally exposed to these metals from the environment by inhalation, in drinking water and contaminated food (Sharma *et al.,* 2008), which end up accumulating in the vital organs and produce free radicals that cause oxidative stress (Hashmi*et al.,* 2007).

The paucity of published data in Africa on heavy metal concentrations in suspected high level sources such as vegetables and other food stuffs on the market would appear to indicate that the problem is wider than anticipated (Agrawal, 2003). One of the common heavy metals is Cadmium (Cd) which is known to be a potent human carcinogen associated with cancers of the lung, prostate, pancreas and kidney (Patra *et al.,* 2011).

The commonly used chelating agents such as British Anti Lewisite (BAL), calcium disodium ethylenediamine tetra acetic acid ($CaNa_2EDTA$), meso 2,3-dimercaptosuccinic acid (DMSA) sodium 2, 3- dimercaptopropane 1-sulfonate (DMPS) are so far the best known management remedies against metal poisoning, although they are associated with grave adverse-effects (Flora *et al*., 2008). Alternative and effective sources of heavy metal poisoning treatment are thus necessary. The role of natural compounds that may augment the innate protection against heavy metal induced toxicity in humans is ripe for investigation (Al-Hashem *et al*., 2009). Furthermore, studies have shown that supplementation of antioxidants along-with nutritional chelating agents provides a better treatment regimen than monotherapy with chelating agents (Flora *et al.,* 2008).

Being a product that is most widely consumed worldwide (Cabrera *et al*., 2006; Kodama *et al*., 2010), tea has gained popularity due to its antioxidant ability. This beverage is now increasingly being employed in the management of hypertension, arteriosclerosis, hypoglycemia and hypocholesterolaemia (Ramadan *et al.,* 2009). Additionally, it is

hypothesized that the effectiveness of tea to manage the effects of heavy metal toxicity is guided by its capability of reducing the levels and the prooxidant effects (Flora *et al.,* 2008). It is further hypothesized that tea might have greater affinity for heavy metals and coupled with the following attributes, it is a good dual antioxidant; low mammalian toxicity, ability to penetrate membranes and even pass through the blood- brain barrier (BBB), its capacity to rapidly eliminate the toxic metal, high solubility in water, its capability to form non-toxic complexes, ease of administration *et cetera,* (Rice-Evans *et al.,* 1995; Brown *et al.,* 1998;Weinreb *et al.,* 2009; Flora *et al.,* 2010).

Studies have also shown that oral consumption of tea reduces cancer risk (Katiyar and Mukhtar, 1996; Yang *et al.,* 1998). These beneficial effects are mainly attributed to teas anti-oxidant and chelating activities for metal ions (Kumamoto *et al.,* 2001). Furthermore, tea has been shown to be more potent than other antioxidants such as vitamins C and E (Rice-Evans *et al*., 1995). Despite such remarkable characteristics strengthened by research findings, there is still paucity of data linking its pharmacological abilities to chelation of heavy heavy metals. It is in line with this that this study was set up to test tea infusions as metal chelators and antioxidants.. The study endeavored to compare the protective effects of Kenyan black tea extracts that are rich in theaflavins and thearubigins with Kenyan green tea extracts that are rich in catechins with that of EDTA on experimentally induced cadmium toxicity in male wistar albino rats. Likewise, the study looked at any deleterious effects caused by the consumption of Kenyan black tea and green tea extracts.

The assumption in this study was that the chelating agents were likely to mobilize toxic metals from different tissue compartments while the antioxidant would reduce the induced oxidative stress and restore the altered biochemical variables, promoting better clinical recovery (Flora *et al*., 2008).

The overall goal of this study was to determine the ability of tea to chelate and/or ameliorate the effects of Cd-induced toxicity in rats. This was accomplished by monitoring the levels of Cd, reduced glutathione (GSH), Thiobarburic acid reactive substances (TBARS), Zinc fingers and homeoboxes 1 (ZHX1), total proteins, alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) as well as pathological changes on the vulnerable organs particularly liver, kidney, testes and the brain in laboratory rats experimentally treated.

1.2 Statement of the Problem

Heavy metal toxicity has been implicated in pathogenesis of many disease conditions that occur through bioaccumulation and production of free radicals. This cause oxidative stress that is detrimental to biomolecules with subsequent carcinogenesis and/or development of neurodegenerative diseases. According to the World Health Organization (WHO), cadmium exposure is of major public health concern. This is due to the fact that cadmium and its compounds are carcinogenic to humans and have been classified as group 1 carcinogens; the strongest assertion of its carcinogenicty. Like most developing countries, Kenya has not been spared of the hazards associated with this metal. In a survey carried out in Kenya, blood samples taken from children who live near the open Dandora waste dump site exhibited unusually high levels of cadmium whose negative effects were linked to negative health effects such as renal failure, gastro-intestinal disorders and respiratory tract irritations such as asthma and bronchitis. Although chelation therapy has been the mainstay treatment against heavy metal induced toxicity, development of molecules that may be categorized anywhere close to an ideal chelator is far from reality. This is due to the adverse effects exhibited by the classical chelators. Furthermore, the management of these conditions is neither readily available nor cost effective when available. Given the importance of heavy metal induced toxicity in the pathogenesis of many health conditions and the complications associated with the classical chelators, the need to fully understand whether tea, a dual antioxidant with both chelating and antioxidant properties, can be used in the management of Cd induced toxicity cannot be overemphasized.

1.3 Objectives

1.3.1 General objective

To evaluate the effect of Cd toxicity in the cellular biosynthetic and antioxidant system including bio-accumulation and the ameliorative affects of tea in male wistar rats.

1.3.2 Specific objectives

i. To determine total polyphenols and levels of heavy metals in tea.
ii. To determine the effects of administered Cd on total proteins, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutathione and zinc fingers and homeoboxes in the serum, brain and liver of rats.

iii. To determine the effect of orally fed tea infusions to ameliorate the effects of injected Cd on total proteins, ALP, ALT, AST, glutathione and zinc fingers and homeoboxes in the serum, brain and the liver of rats.

iv. To determine the levels and effects of Cd in the histology of the liver, brain, testes and the kidney of rats.

1.4 Hypotheses

i) There are low levels of total polyphenols and no heavy metals in tea.

ii) Administered Cd does not have an effect on total proteins, ALP, ALT, AST, GSH, TBARS and ZHX1 in the serum, brain and liver of rats.

iii) Orally fed tea infusions do not ameliorate toxic effects of injected Cd proteins, ALP, ALT, AST, glutathione and zinc fingers and homeoboxes in the serum, brain and the liver of rats.

iv) Administered Cd neither accumulates nor have an effect on the histology of the liver, brain, testes and the kidney of rats.

1.5 Justification

There is a dire need for enhancement of treatment and management of heavy metal toxicity. The currently used chelating agents have been linked to severe adverse-effects such as depletion of essential metals, inability to pass through the cellular membranes, redistribution of the metals to body organs and augmentation of oxidative stress. Since bio-accumulation of metals and oxidative stress plays a central role in heavy metal induced toxicity, it is rational to suggest that, antioxidant and chelation therapy using tea might be important in ameliorating this menace. Tea, a widely consumed beverage is non-toxic and is endowed with metal chelating and antioxidant properties besides its capability of crossing the blood brain barrier (BBB). Therefore, it is likely to be best suited for management of heavy metal induced toxicity.

CHAPTER TWO
LITERATURE REVIEW

2.1 Heavy Metals

Heavy metals are chemical elements that have a relatively high density usually > 5g/cm^{3}and are toxic or poisonous at low concentrations (Alissa *et al*., 2011; Tchounwou*et al*., 2012). They include cadmium, lead, arsenic, aluminum among others (Nieboer *et al.,* 1980). Highly toxic heavy metals are environmentally and occupationally widespread pollutants which have been associated with mutagenic, carcinogenic, and teratogenic effects (Stohs *et al.,* 1995; Valko *et al.,* 2005; Cuypers *et al.,* 2010). They have also been shown to stimulate reactive oxygen species (ROS) formation and production and cause injury to the cells (Strydom *et al*., 2006). Though heavy metals act through diverse mechanisms; much of their toxicity is caused by an induction of free radicals, either directly or by poisoning cellular antioxidant enzymes (Ercal *et al.,* 2001).

Cd is among the most widespread pollutants that have been known to be a dangerous occupational hazard and also an environmental toxin of public health concern (Al-Hashem *et al*., 2009). It is an industrial pollutant, arising primarily from battery, electroplating, pigment, plastic, fertilizer industries, and cigarette smoke (Valko *et al.,* 2005). Further, it is a known human carcinogen whose underlying mechanisms of action are still vague (Aimola *et al*., 2012).

2.2 Cadmium Toxicity

Cadmium is an extremely poisonous nonessential heavy metal that is well known for its undesirable influence on the biological enzymes of the cellular systems, oxidative stress and for causing nutritional deficiency in plants (Irfan *et al.,* 2013). Plants progressively take up Cd which mount up and concentrate along the food chain, and eventually reach the human body. Alarmingly, global statistics show that more than 500,000 workers get exposed to toxic cadmium each year as per the Agency for Toxic Substances and Disease Registry (Bernard, 2008; Mutlu *et al.,* 2012). Additionally, environmental cadmium exposure in Japan and China is comparatively higher than in any other country; with the total area contaminated by cadmium in China being more than 11,000 hectares and its yearly sum of manufacturing waste of cadmium released into the environment assessed to be more than 680 tons (Han *et al.,* 2009). Cadmium is primarily found in fruits and vegetables due to its high rate of soil-to-plant transfer (Satarug *et al.,* 2011).

Cd toxicity relates to smelting where the main route of exposure is through the lungs (Gaurav *et al.,* 2010). Furthermore, its vulnerability is aggravated by the fact that humans consume both plants and animals that absorb Cd efficiently and concentrate it within their tissues (Stohs *et al.,* 1995). Numerous studies have demonstrated that Cd stimulates free radical production, resulting in oxidative deterioration of lipids, proteins and deoxyribonucleic acid (DNA) (Kumar *et al*., 2012). This initiates various pathological conditions in humans and animals (Valko *et al*., 2005). Being a potent human carcinogen, it has been associated with cancers of the lung, prostate, pancreas and kidney (Koedrith *et al.*, 2011). This condition is further aggravated by its long biological half-life of 15 to 30 years, making its excretion nearly impossible (Djukić-Ćosić *et al.,* 2008). Although the specific molecular events accompanying Cd induced transformation are elusive, it is assumed that its poisoning is associated with a complex onco-genetic mechanism, in which more than a single pathway might be implicated (Aimola *et al*., 2012). Since it is not an essential trace element; it does not have a metabolic pathway (Khan *et al.,* 1992). Therefore, once it is incorporated, net accumulation occurs in the tissues where it interacts with other metals such as iron, calcium, copper as well as zinc and interferes with the enzyme activities of metabolic pathways in addition to depleting the endogenous antioxidant system (Valko *et al*., 2011). In animal diets, the maximum concentration of cadmium tolerated is 0.5mg/kg (McDowell, 1992; NRC, 2001).

Various mechanisms have been proposed on how heavy metals are able to generate free radicals either directly or indirectly (Migula *et al.*, 2004). Fenton and Haber-Weiss reactions are the most common mechanisms that cause generation of free radicals such as superoxide and hydroxyl radicals (Valko *et al.*, 2011; Padhy *et al.*, 2013). These radicals end up damaging DNA, causing lipid peroxidation and protein modification. Evidence from studies have shown that cadmium displaces iron and copper from the biological system, thus increasing intracellular levels of the two metals which then undergo fenton and Haber-Weiss reactions (Valko *et al.,* 2011). Subsequently, the link between oxidative damage and carcinogenesis of metals cannot be overlooked since most DNA base modifications caused by free radicals are pro-mutagenic (Valko *et al.,* 2005; Attia *et al.,* 2010).

2.3 Absorption of Cadmium

Extensive research has shown that Cd is absorbed by intestinal cells and transported by blood flow to the liver where it induces metallothionein synthesis, a protein that is involved in detoxification (Yang *et al*., 2015), forming Cd-metallothionein complex that is toxicologically inert (Djukić-Ćosić *et al.,* 2008). The complex is released into the bloodstream and filtered by

kidney glomeruli, where it can be degraded by lysosome enzymes from kidney tubular cells (Roman *et al.,* 2002). Since metallothionein action is limited (Stanevičinė *et al.,* 2008), when animals ingest excessive doses of Cd of 1 μg/kg/ day, it accumulates in the organs for a long time, probably for decades causing sub-acute, acute or chronic poisoning (Djukić-Ćosić *et al.,* 2008) which cause severe damage to various organs such as the liver, kidney, lungs, nervous system, testes, intestine and bones (NRC, 2001; Alonso *et al.,* 2004; Newairy *et al.,* 2007).

2.4 Cadmium and Oxidative Stress

Cd is one of the exogenous sources shown to indirectly produce ROS which accumulate in various cell lines (Szuster *et al.,* 2000; Alisa *et al.,* 2011) causing oxidative stress (Figure 1) and inhibiting the electron transfer chain in the mitochondria (Wang *et al.,* 2004). However, unlike other heavy metals, Cd is unable to generate free radicals by itself (Galan *et al.,* 2001). Several studies have explained the indirect role of Cd in free radical generation (Renugadevi *et al.,* 2009), proposing that it can replace iron and copper (Figure 1) in various cytoplasmic and membrane proteins such as ferritin and apoferritin, thus increasing the amount of unbound free or chelated copper and iron ions participating in oxidative stress via Fenton reactions (Casalino *et al.,* 1997).

2.5 Effects of Cadmium on ZHX1

Zinc fingers and homeoboxes gene family are nuclear homodimeric transcriptional repressors that interact with the A subunit of nuclear factor -Y (NF-YA), contain two C2H2-type zinc fingers and five homeobox DNA-binding domains, heterodimerizes with members 2 and 3 of the zinc fingers and homeoboxes family and are encoded by the *ZHX1* gene (Yamada *et al*., 1999a, b). These proteins contain homeodomains (HDs) as well as 2 cysteines and 2 histidines (C2H2)-type zinc finger motif which is the main DNA-binding proteins in eukaryotic cells (Parpworth *et al.,* 2006). They also contain basic and acidic regions (Yamada *et al.,* 2002); which function as the DNA binding domain (DBD) for nuclear localization signal (NLS) and transcription, correspondingly (Gehring *et al.,* 1994; Philipsen *et al.,* 1999). ZHX1 is one of the biochemical targets that Cd inhibits in eukaryotes (Verbost *et.al.,* 1988). The proposed mechanisms of zinc finger interference by Cd are either isostructural substitution, replacement with altered geometry, mixed complex formation or catalysis of thiol oxidation (Hartwig, 2001). Hanas *et al.,* (1996) affirms that cysteine rich regulatory proteins such as ZHX1 or Transcription factors IIIA (TFIIIA) involved in signal transduction are potentially at risk of Cd inhibition *in-vivo* especially at low sub-lethal concentrations. Since these factors are involved

in regulatory transcription responsible for cell growth or differentiation, their inhibition by Cd could alter normal cell growth and cause teratogenesis rendering Cd a tumor promoter (Hanas *et.al.,* 1996). Research has strongly indicated that Homeobox genes are nuclear proto-oncogenes that mutate to oncogenes inducing cell transformation *in-vitro* (Gehring *et al.,* 1994). Furthermore, zinc finger proteins have been described as DNA binding motifs in transcription factors and have been identified in several DNA repair enzymes. Metals such as Cd, arsenic and nickel have been shown to inhibit nucleotide excision repair (NER) and base excision repair (BER) (Hartwig, 1998). It has been shown that the metals modify gene expression by interfering with signal transduction pathways that play very essential roles in cell growth and development resulting to cancer phenotype (Valko *et al.,* 2006). These metals deregulate cell proliferation by activating various transcription factors that are known to control cell cycle progression and apoptosis (Evan and Vousden, 2001). It is also notable that, if these proteins are ubiquitously expressed, their involvement in the formation of highly organized nucleoprotein complexes that participate in the regulation of numerous genes in various tissues (Yamada *et al.,* 2002) cannot be overemphasized.

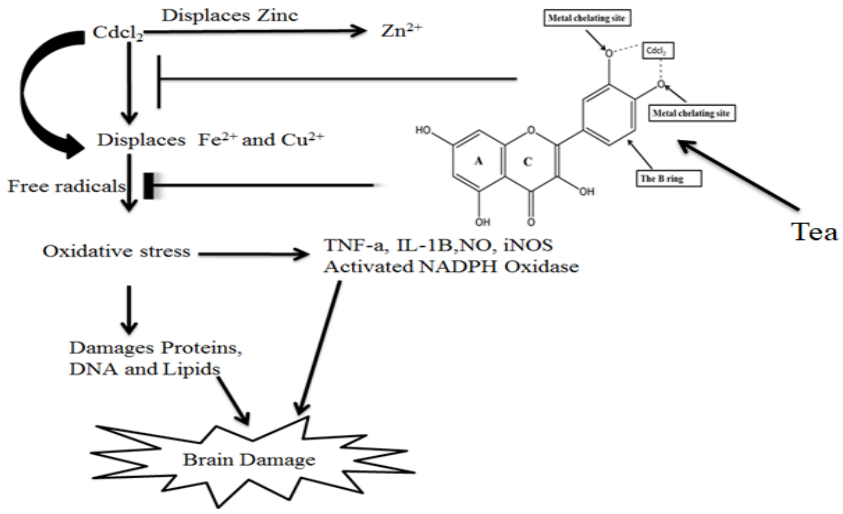

Figure 1: Mechanisms of action of tea polyphenols during heavy metal toxicity

2.6 Effect of Cd on GSH levels

Glutathione (GSH) coordinates the body's antioxidant defense mechanisms (Abolfathi *et al.,* 2011). The regulation of diverse functions of GSH and the precise mechanisms by which it acts as a signal transducer during heavy metal induced toxicity is however still elusive (Jozefczak *et al.,* 2012). Perturbation of GSH status in the cell leaves the biological system susceptible to oxidative stress. Previous research has reported that exposures to high Cd levels typically result

in GSH depletion (Regoli and Principato, 1995; Ringwood *et al.,* 1998; Mitchelmore *et al.,* 2003; Toplan *et al.,* 2003), whereas at lower Cd concentrations no change or an increase in GSH levels can be observed (Viarengo *et al.,* 1990; Ringwood *et al.,* 1999; Mitchelmore *et al.,* 2003; Regoli *et al.,* 2004; Lannig *et al.,* 2006). Furthermore, Cd toxicity increases Glutathione S transferases (GST) activity, glutathione peroxidase (GPx) and catalase activity (Vazquez-Medina *et al.,* 2010), causing an increase in the rate of H_2O_2 removal, which is the principal substrate for the antioxidant enzymes (Brown *et al.,* 1988). Although GSH does not react directly with hydroperoxides, its use as a substrate for glutathione peroxidase has been shown to be the predominant mechanisms for reduction of H_2O_2 and lipid peroxides (Rhee *et al.,* 2005). The modulation of H_2O_2 levels by peroxide removing enzymes is essential for the actions of H_2O_2 as a second messenger in the activation of nuclear factor erythroid 2–related factor 2(NrF2) pathway which leads to the upregulation of proteins involved in GSH synthesis, antioxidant defense and phase II detoxification via the antioxidant response element (ARE) (Sen and Packer, 1996; Rhee *et al.,* 2005). Moreover, the rate limiting step of GSH biosynthesis is catalyzed by glutamate-cysteine ligase (GCL) which is regulated by firstly; feedback inhibition by GSH (Noctor *et al.,* 2012), secondly, metal induced stress during *de novo* synthesis of GSH through redox sensitive repressor-binding protein, and finally by post translational redox controls (Noctor *et al.,* 2002; Cuypers *et al.,* 2012). Studies have shown that GCL in plants forms a homodimer linked by two redox-sensitive disulfide bonds, causing a conformational change that significantly inactivates the enzyme and thus upregulates GSH synthesis in response to metal induced oxidative stress (Hothorn *et al.,* 2006; Gromes *et al.,* 2008). Since feedback inhibition of the rate limiting enzymes is one of the regulation mechanisms during GSH biosynthesis, then depletion of GSH by cadmium would possibly be an important mechanism driving accelerated rates of GSH biosynthesis.

2.7 Effect of Cd on Lipids

Ayala *et al.* (2014) defined lipid peroxidation (LPO) as the reaction of oxidative deterioration of membrane polyunsaturated fatty acids. It is radical chain of reactions whereby hydrogen atoms are distracted from unsaturated fatty acids, giving rise to alkyl radicals that react at near diffusion limited rates with molecular oxygen to come up with lipid hydroperoxyl radicals (Lambart and Elias, 2010). This latter form is associated with cellular damage as a result of the aldehydes such as malonaldehyde (MDA) that are formed when lipid hydroperoxides break down in the biological systems (Siddique *et al,* 2012). It is well documented that cadmium exposure is associated with elevated lipid peroxidation in various

tissues such as lung, brain, kidney, liver, erythrocyte and testes (Manca *et al.,* 1991; Sarkar *et al.,* 1997). Studies have shown that cadmium through diverse routes causes lipid peroxidation in membranes of erythrocytes and tissues such as the liver, kidney, brain and testes where an elevation of MDA is a clear indicator of oxidative damage (Gutteridge, 1995). Equally, MDA may be a biomarker for osteoclastic activity since it causes bone loss (Akpolat *et al.,* 2013). Other mechanisms through which cadmium toxicity causes lipid peroxidation (LPO) in various tissues are induction of phagocytic cells that produces reactive species (Stohs and Bagachi, 1995), displacement of iron and copper from its binding sites leading to acceleration of free radicals (Casalino *et al.,* 1997).

2.8 Role of Cd on tissue histology and histopathology

Studies have shown that Cd bioaccumulation in the liver and kidney of rats causes severe changes in their histology and functionality (Kjellström, 1986; Mitsumori *et al*., 1998). Early exposure causes interstitial fibrosis, tubular necrosis, and glomerular epithelial cell hypertrophy (Aughey *et al*., 1984). The liver is the principal target of Cd poisoning causing ruthless hepatocyte necrosis, fatty changes and inflammatory cell infiltrations (Renugadevi and Prabu, 2010). Furthermore, Cd toxicity is linked to lipid peroxidation in various tissues (Sarkar *et al*., 1997)

2.9 Interactions between Cd and essential metals

Cd poisoning is mainly based on ionic mimicry which happens by its replacement of essential minerals such as calcium, zinc, copper and iron (Choong*et al*., 2009). This leads to protein mis-folding or unfolding and malfunction leading to necrosis (Gardarin *et al*., 2010).Therefore, the fundamental basic mechanisms of Cd toxicity can be summed up as the interactions between Cd and essential metals (Vesey, 2010) and the oxidative stress caused by Cd exposure (Farmand*et al*.,2005; Liu *et al*., 2009). Additionally, these mechanisms are still interconnected because the metabolic disorder of essential mineral such as zinc, calcium and selenium also induces adverse effects in the oxidative and anti-oxidative systems (Brenneisen *et al*., 2005) by altering essential minerals homeostasis (Hirano *et al*., 1991).

2.10 Chelation Therapy

Currently, there is no effective treatment for cadmium toxicity, except supportive treatment according to the symptoms of intoxication. Studies have recommended chelation therapy as a therapeutic strategy for dissipating heavy metals from the biological system through binding and formation of complex structures which are easily excreted from either

intracellular or extracellular spaces (Andersen, 1999; Klaassen, 2006; Bradberry *et al*., 2009; Renugadevi *et al*., 2009; Flora *et al*., 2010), and reduction of oxidative stress (Andersen, 2004). However, the most commonly used chelation modulators such as EDTA and dimercaptosuccinic acid (DMSA) (Boscolo *et al.,* 2005; Bradberry *et al*., 2009; Mikirova *et al*., 2011) are compromised, with various side effects especially loss of essential elements, having numerous adverse effects, non-specific binding and administration inconvenience (Flora *et al.,* 2008). Although a range of metal chelators are now available for toxic metal chelation, development of molecules that may be categorized anywhere close to an ideal chelator is far from reality (Flora *et al.,* 2010). Nevertheless, studies have shown that co-administration of nutritional antioxidants along-with a chelating agent prove to be a better treatment regimen than monotherapy with chelating agents (Flora *et al.,* 2008). Thus this calls for an urgent need for alternative safe and effective treatment against heavy metal induced toxicity.

2.21 Types of tea and their distribution in Kenya

Tea (*Camellia sinensis)* belongs to the family Theaceae (*Camelliaceae*). Three types of *Camellia* with stimulant properties developed separately are grown in Kenya namely *Camellia sinensis* Var. *sinensis, C. sinensis* Var. *assamica* (Anonymous, 2002) and the Cambodian type (Anonymous, 2003). Tea was first introduced in Kenya from India by a colonial settler G.W Caine in 1903 (FAO, 2001). Currently tea is the leading export crop in Kenya (IARC-WHO, 1991). It is mainly grown in Kericho, Bomet, Nandi, Kiambu, Thika, Maragua, Muranga, Sotik, Kisii, Nyamira, Nyambene, Meru, Nyeri, Kerinyaga, Embu, Kakamega, Nakuru and Trans-nzoia. In these areas the crop enjoys 80% favorable weather patterns (Wachira, 2002).

2.22 Isolation and characterization of tea polyphenols

Tea is a widely consumed non-toxic beverage that is rich in polyphenols. (Cabrera *et al* 2006). The isolated polyphenols include but not limited to catechins, theaflavins (TF),thearubigins and anthocyanins and are thought to contribute to its health benefits by acting as antioxidants directly through scavenging free radicals and chelating redox-active transition metal ions (Karori *et al*., 2007; Kerio *et al*., 2013), The polyphenols indirectly hinder the redox-sensitive transcription factors, nuclear factor-kappaB (NF-kB) and activator protein-1(AP-1) as well as inhibition of "pro-oxidant" enzymes, such as inducible nitric oxide synthase, lipoxygenases, cyclooxygenases, xanthine oxidase (Yang *et al*., 2002) and induction of phase II antioxidant enzymes, such as glutathione S-transferases and superoxide dismutases (Cabrera *et al*., 2006). Tea polyphenols may also play a significant role as a naturally occurring

antioxidant substitute consequently contributing to human health (Karori *et al.,* 2007). Recent findings have shown that tea polyphenol administration inhibits carcinogen-induced increases in the oxidized DNA base, 8-hydroxy-2'-deoxyguanosine (Frei *et al*., 2003). Theaflavins (TF) which are dimers of catechins have more hydroxyl (OH) groups, which are considered to be necessary for exerting radical scavenging activity and thus have more antioxidant properties than do catechins alone (Skrzydlewska *et al.,* 2005).

Extensive research demonstrates that acute and chronic human intoxications with a wide range of metals can be treated with considerable efficiency by co-administration of an antioxidant whether natural or synthetic with a chelating agent or supplementation of antioxidants along-with a chelating agent (Flora *et al.,* 2008). Furthermore, tea polyphenols have been shown to readily cross the blood brain barrier (BBB) **(**Rashid *et al*., 2014**;** Weinreb *et al*., 2009) and to enhance brain antioxidant activity. This observation makes tea even more superior to other chelating agents since it may have the capacity to capture and isolate intracellular metal bioaccumulation in affected tissues. It is in line with this and recent literature that strengthens the perception that diverse molecular signaling pathways participate in the neuroprotective and cytoprotective activities of tea polyphenols rendering this natural compound the ability to reduce the risk of various conditions caused by heavy metal induced toxicity (Rice-Evans *et al.,* 1995). Although tea may confer its protection through scavenging of free radicals (Spencer *et al.,* 2001; Spencer, 2007), studies have shown that it may also perform its therapeutic actions by modulating P13 kinase/Akt, tyrosine kinase, protein kinase C (PKC), and mitogen activated protein kinase (MAP kinase) signaling pathways, suppressing JNK and downstream partiners c-jun and pro-caspase-3 (Schroeter *et al.,* 2001; Kerio *et al*., 2011). Tea also attenuates microglia and astrocyte mediated neuroinflammation through inhibition of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) expression, nitric oxide (NO) production, cytokine release and NADPH oxidase activation and subsequent generation of ROS in gliosis (Vauzour, 2012). This is made possible by inhibition of p38 and or ERK1/2 which have been shown to regulate iNOS and TNF-alpha in activated glial cells (Bhat *et al.,* 1998). This may influence downstream pro-inflammatory transcription factors such as nuclear factor Kappa B (NF-kB) which responds to p38 signaling and is involved in iNOS induction (Bhat *et al.,* 2002; Karori *et al*., 2007). These findings and the multifactorial antioxidant-chelating effects of tea extracts strongly indicate that tea may be protective against conditions that arise from cadmium induced toxicity where oxidative stress may be implicated. Since strong evidence has now emerged showing that tea is a dual antioxidant with metal

chelating properties, there is a robust scientific rationale for testing it as a potential therapeutic agent that can be used to improve treatment outcome of heavy metal induced toxicity.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Tea Samples and Processing

Processed tea (*Camelia sinensis*) from cultivar TRFK 6/8 was used in this study. The tea cultivar was cultivated at the Tea Research Institute (TRI), Timbilil Estate in Kericho (latitude 0°22'S, longitude 35°21'E, altitude 2180m above sea level). Both black and green tea samples were processed from the cultivar at the Tea Research Institute(TRI) miniature factory using standard TRI optimized manufacturing procedures.

Freshly picked two leaves and a bud were used for the manufacture of both black (aerated) and green (unaerated) teas. For the processing of black tea, samples were physically withered for 18 hours to attain a moisture content of 50-65%. Maceration was then done using a Cut Tear and Curl (CTC) (Williamson Tea, UK) machine followed by aeration at 24ºC for 1-2 hours in an aeration cabinet (Tea Craft, Bedford, UK). Finally, the tea samples were dried for 20-25 minutes at 120ºC in a fluid bed drier (Tornado 501 Sherwood, Tea Craft, UK) to attain a moisture content of 4.5%. For the processing of green tea, leaf samples were steamed (Philips HD9120, China) at 90ºC for 1 minute, macerated using a CTC machine and dried in a fluid bed drier (Tornado 501 Sherwood, Tea Craft, UK) at 120ºC for 20-25 minutes. The tea samples were stored in sealed silver lined sachets and stored at room temperature at the TRI tea and health laboratory until use (Ambadekar *et al*., 2012).

3.2 Sample Treatment for Polyphenol and Catechin Analysis

Polyphenol and Catechin analysis were carried out as previously described by Koech *et al.,* (2014). Two grams of the pulverized samples were placed on a pre-weighed moisture free dish and placed in an oven for 16 hours at 103ºC to dry for the determination of dry matter. Of this, 0.2g was weighed into an extraction tube. Five milliliter of hot 70% methanol/water (v/v) was then dispensed into the sample as an extraction mixture and vortexed. Heating of the extraction tube was continued in the water bath for 10 minutes with mixing in the vortex mixer after every 5 minutes. The samples were then centrifuged at 3500 revolutions per minute (rpm) for 10 minutes followed by decantation into a graduated tube and the extraction procedure repeated. The extracts were combined and made up to 10ml with cold methanol/water mixture. One milliliter of the sample extract was then transferred into a graduated tube and diluted to 5ml with stabilizing solution (10% v/v acetonitrile with 500µg/ml EDTA and ascorbic acid). The solution was then filtered through a 0.45µm nylon membrane filter. A 20µl aliquot of this solution was injected into the HPLC machine for analysis as described by Karori *et al.,* (2007).

3.2.1 Determination of Catechins in Tea Infusions

HPLC method was used to assay for the tea catechins as described by Kerio *et al.,* (2012). Briefly, A Shimadzu LC 20 AT HPLC system fitted with a SIL 20A auto sampler and a SPD-20 UV visible detector with a class LC 10 chromatography work station was used for analysis of the prepared samples (Shimadzu, Japan). A Gemini 5µM C18- Phenyl, 250mm x 4.6mm (Phenomenex, Torrance, CA, USA) separation column with a Reodyne precolumn filter disk was used. The sample was then degassed before injection into the HPLC system. A gradient elution was then carried out using two solvent systems. Mobile phase A consisting of acetonitrile/acetic acid/double distilled water (9/2/89 v/v/v) and mobile phase B consisting of acetonitrile/acetic acid/double distilled water (80/2/18 v/v/v). The mobile phase composition for a binary gradient condition started at 100% solvent A for 10 minutes then over 15 minutes a linear gradient to 68% mobile phase A, 32% mobile phase B and held at this composition for 7 minutes. The condition was then reset to 100% mobile phase A and then allowed to equilibrate for 10 minutes before the next injection.

The identification of individual catechins was then carried out by comparing the retention times and UV-absorbance of sample peaks with peaks obtained from the mixed known catechin standards under the same conditions. Pure Catechin (C), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) standards purchased from Sigma Aldrich UK were used. The quantification of catechins was performed at 278nm and the total catechin as a percentage by mass on a sample dry matter basis was given on the summation of individual catechins as shown in the formula provided below;

% Total catechins = [%ECG + %EC + %EGCG + %C] content

The determinations were done in triplicate.

3.2.2 Determination of Total Polyphenols in the Tea Infusions

The Folin-Ciocalteu phenol reagent method was used to determine total polyphenols as described by Pourmorad *et al.,* (2006). One milliliter of the sample extract was transferred to a volumetric flask and diluted to a final volume of 100ml with distilled water. One milliliter of the diluted sample extract was transferred in duplicate into separate tubes. Five milliliters of 10% (v/v) dilute Folins-Ciocalteu was then pipetted into each tube and mixed. Within 3-8 minutes after addition of the Folins-Ciocalteu phenol reagent, 4ml of 7.5% w/v sodium carbonate solution was added to each tube, stoppered and mixed well. The mixture was then

allowed to stand at room temperature for 60 minutes and then optical densities (OD) measured using a CE 393 Cecil digital grating spectrophotometer set at 765nm (Cecil Instruments, U.K). A calibration curve was obtained for gallic acid over a concentration range of 10µg/ml to 60µg/ml. The OD readings of the test of the samples were referenced to the calibration curve to determine the total polyphenols content of the tea samples using a formula outlined below;

$$m = \frac{m_o \times V \times W_{DM,\,std} \times 10000}{100 \times 100}$$

Where;

m_o- is the mass of Gallic acid monohydrate (g) used to prepare the stock standard solution

V - is the volume of Gallic acid stock standard solution (g) used to prepare the standard solution labelled A, B, C, D and E

$W_{DM,}$ std – is the dry matter content, expressed as a mass fraction (%) of the Gallic acid

The total polyphenol content expressed as percentage by mass on sample dry matter basis was calculated using the formula:

$$W_T = \frac{(D_{sample} - D_{intercept}) \times V_{sample} \times d \times 100}{S_{std} \times M_{sample} \times 10000 \times W_{DM,\,sample}}$$

Where;

D_{sample} – is the optical density obtained for the sample test solution

$D_{intercept}$ – is the optical density at the point of the best fit linear calibration

M_{sample} – is the mass (g) of the sample test portion

V_{sample} – is the sample extraction volume in milliliters (10ml for leaf tea)

D – is the dilution factor used prior to the calorimetric determination

$W_{DM\,sample}$ – is the dry matter content (expressed as mass fraction in percent) of the test sample

The determinations were done in triplicate.

3.2.3 Analysis of Total Theaflavins Content in the Tea Infusions

Total theaflavins (TF) were determined as described by Hilton and Palmer Jones (1973). Nine grams of the tea samples were weighed and infused with 375ml of boiling water in tared flask. The flask was then agitated in a mechanical shaker for 10 minutes. The infusion was filtered and allowed to cool to room temperature. Ten milliliters of the tea infusion was then mixed with 10ml of isobutylmethylketone (4-methylpenta-2-one, IBMK) and the mixture shaken for 10 minutes before being allowed to stand at room temperature (RT) for layer separation. Two milliliters of the upper layer was pipetted into a test tube followed by 4ml

ethanol and 2ml flavognost (2g diphenyl boric acid -2-aminoethyl ester dissolved in 100ml of ethanol). The contents were mixed and allowed to develop green colour. The absorbance was read in triplicate for each sample using a CE 393 Cecil digital grating spectrophotometer set at 625nm (Cecil Instruments, U.K). Total theaflavins were then calculated by the formula outlined below;

TF (µmol/g) = A 625 x 47.9 x100/DM ; where A 625 is the absorbance at 625nm and DM is the dry matter of the sample.

3.2.4 Determination of Total Thearubigins in Tea Infusions

The total thearubigins was determined as described by the methods of Roberts and Smith, (1961). A 1% v/vaqueous solution of anhydrous disodium hydrogen orthophosphate (6.0 ml) was added to the 6.0 ml of cooled tea infusion. The resulting mixture was extracted with ten milliliters of ethyl acetate by vigorous shaking for one minute. The mixture was allowed to settle and the aqueous layer drained off. 5.0 ml of ethyl acetate was then added to the ethyl acetate extract containing the theaflavin fraction in the separating funnel. The ethyl acetate extract (10 ml) was then diluted to 25 ml with methanol in 25 ml volumetric solution (solution E_1). The tea infusion (1.0 ml) was mixed with 9.0 ml of distilled water and made to 25 ml in a volumetric flask with methanol (solution E_2). Saturated aqueous 10 % oxalic acid (1.0 ml) was then added to 1.0 ml of tea infusion and 8.0 ml of distilled water and made to 25 ml with methanol (solution E_3). The absorbance of solutions E_1, E_2 and E_3 were obtained at 380 nm and 460 nm respectively using a Shimadzu UV-1800 series spectrophotometer (Shimadzu, Japan) with distilled water as the blank. Each sample was extracted in duplicate for the determination of theaflavin (TFs), thearubigin (TRs) fractions and brightness levels. The percent TFs and TRs values were then calculated using the formulae;

$$TFs\% = 2.25 \times E_1 \times DM$$

$$TRs\% = 7.06 \times (4E_3 - E_1) \div DM\ \%$$

3.2.5 Free Radical Scavenging Activity of Tea Samples

The stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was used for determination of free radical scavenging activity of the tea samples using a modified method of Brand-Williams *et al.*,(1995). The assay is based on the measurement of scavenging ability of antioxidants towards the stable DPPH radical (Moreno, 2002). Five grams of tea were infused in 100ml of boiling doubled-distilled water followed by magnetic stirring and additional

steeping for 30 minutes at room temperature (RT). The extracts were strained through a nylon mesh (120µm) followed by a filter paper (Whatman No. 54). Aliquots of the extracts were kept frozen at -18°C until further use. The soluble solid extracts were standardized to give stock solutions of 50mg soluble solids per 100ml of 50% methanol. A 50µl methanolic solution of tea samples was placed in a cuvette and 2ml of 6mM DPPH in 80% methanol added. The absorbance was read at 517nm using model CE 393 digital grating spectrophotometer (Cecil Instruments, U.K) in intervals of between 15 and 30 minutes before the reaction reached a plateau phase. The percentage inhibition of the DPPH radical was then calculated from the absorbance data as outlined below;

% inhibition against DPPH = [(AB-AA)/AB] x 100;
Where;
AB is the absorbance of the blank sample (50µl double distilled water and 2ml DPPH) and AA is the absorbance of the tested sample after 15 minutes.
The determinations were done in triplicate.

3.2.6 Determination of Heavy Metals in Tea Infusions

Heavy metal determination in the tea samples was carried out as outlined by Ambadekar, (2012). Briefly, 0.5g of the pulverized test sample was transferred into a beaker, mixed with 10ml of nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a ratio of 4:1 and then macerated overnight. The sample was then heated up and boiled to a brownish black color. The above mixture was added and continuously heated till the solution became clear to transparent. The temperature was then raised and heated continuously till a thick white smoke dispersed. The slaked solution which became clear and transparent was left to cool and then transferred into 50ml volumetric flask. A solution of 2% nitric acid was added to the volumetric flask containing the sample to the 50ml mark and shaken well to dilute the sample. Similarly blanks were treated using the same acids to minimize error during the analysis. Finally, the metal analysis was performed on a flame Atomic Absorption Spectrometer (AAS) (Varian Spectra AA 880, Australia). Standard curves were constructed with metal solutions of known concentrations. The unknown samples were then analyzed against the standard curve for measuring the concentration of the desired metal. The concentrations were expressed in microgram/gram of the sample.
The determinations were done in triplicate.

3.3 Experimental Rats

Fourty, six months old male wister rats weighing between 300-400g were obtained from the University of Nairobi-Chiromo animal house. Experimental procedures and code of ethics involving experimental animals were reviewed and approved for adherence to ethical standards by the Institutional Animal Care and Use Committee (IACUC) of the Institute of Primate Research (IPR). All rats were treated with 0.01ml of Ivermectin (Ivermectin®, Anupco, Suffolk, England) at a dose of 0.2mg/kg body weight injected subcutaneously to each mouse to eradicate endoparasites and ectoparasites infestation. The rats were maintained in standard rat cages on a 12-hour light–dark schedule at a temperature of 23°C and provided *ad libitum* access to standard rat cubes (Unga feeds Ltd, Kenya) and drinking water. The rats were maintained under the conditions described above for 2 weeks to acclimatize them before the experimentation started.

3.4 Experimental Design

Following acclimatization for two weeks, a randomized block design was set up where the rats were randomly divided in eight groups of 5 rats each to represent the different treatments as shown in table 1. A volume of 6ml oftea infusions and 3ml of ethylenediamine tetra acetic acid (EDTA) were given orally by gavage at a dosage of 400mg/kg and 200mg/kg body weight (bwt) respectively for 52 days. A separate group of control animals were also orally supplied with distilled water by gavage for the same duration. Additionally, 0.3ml salt of cadmium chloride ($CdCl_2$) which was reconstituted in distilled water was administered subcutaneously at a dosage of 2mg/kg bwt for 42 days (Gaurav, 2010).

After lapse of the experimental period of 52 days, carbon dioxide was used to euthanize the animals after which blood was drawn from the heart by cardiac puncture using vacutainer needles. A volume of 5ml blood was collected from each rat and put into ant-coagulated purple capped vacutainer^{BD} tubes for hematological examinations and 5ml collected into red capped vacutainer^{BD} tubes for serological analysis. Liver, testes, brain, kidney, spleen and bones were also excised, snap frozen in dry ice and stored in liquid nitrogen until required for analysis.

Table 1: A representation of the study design

Group	No. of rats	Treatment	Dosage
I	5 rats	D.H_2O (vehicle)	6ml
II	5 rats	Na_2EDTA	200mg/kg bwt
III	5 rats	ABTE	400mg/kg bwt
IV	5 rats	AGTE	400mg/kg bwt
V	5 rats	$CdCl_2$	2mg/kg bwt
VI	5 rats	$CdCl_2$+ Na_2EDTA	2mg/kgbwt+200mg/kg bwt
VII	5 rats	$CdCl_2$+ ABTE	2mg/kg bwt + 400mg/kg bwt
VIII	5 rats	$CdCl_2$+ AGTE	2mg/kg bwt + 400mg/kg bwt

AGTE- Aqueous green tea extracts, ABTE- Aqueous black tea extracts, bwt-body weight, D.H_2O Distilled water

3.4.1 Determination of Packed Cell Volume (PCV) and Body Weight

Packed cell volume (PCV) was determined at one week interval as described by Achuba (2008). Blood was taken from each mouse by tail snip into heparinized capillary tube to obtain approximately 75µl of blood. After collection, the tube was sealed with plasticin on the sucking end and centrifuge using microhamatocrit II centrifuge at 12000 rpm for 5 minutes to fractionate whole blood into cellular and plasma components. The hematocrit reading was manually done using a micro hematocrit reader and expressed as a percentage of the total blood volume. The body weight of each rat was also determined at one week interval using the analytical electronic balance (Mettler PM34, Doltarange®).

3.4.2 Tissue Homogenization

Snap frozen whole organs were mechanically homogenized on ice cubes (4^oC) in a solution containing 0.5mls of 0.25M sucrose, 5mM Hepes-Tris, pH 7.4 with protease inhibitor cocktail to a final concentration of 10% (w/v). The homogenates were then aliquoted in triplicates into 1.5ml cryovials to avoid repeated freeze thaw process and then stored at -80 ^oC until analysis.

3.4.3 Determination of Reduced Glutathione

Glutathione assay was performed as described by Rahman *et al*. (2007) with modifications from Rashid *et al.,* (2014). A volume of 50µl of tissue homogenates was mixed with a 50µl solution containing sulphosalicylic acid (SSA) (5% w/v) and 0.25mM EDTA. The mixture was then centrifuged at 8000rpmfor 10 minutes at 4^oC. Thereafter, a solution

containing 200µmol/l GSH standard in 0.5% SSA was prepared and serially diluted in the same diluent to a final concentrations of 100, 50, 25, 12.5, 6.25, 3.13 and 1.56µmol/l, respectively. Ellman's reagent (5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) was prepared by dissolving in 0.1M potassium phosphate buffer with 5mM EDTA disodium salt, pH 7.5) (KPE buffer) to a final concentration of 0.6mg/ml. A volume of 25µl of each standard were loaded on a 96-well microtitre plate to wells B–H in column 1, 2 and 3 followed by 25µl of the sample to the remaining wells in triplicate. To each well, 100µl of freshly prepared DTNB was then added and the absorbance measured at 405nm at intervals of 30 seconds for a period of up to 2 minutes using a multi-detection Dynatech MRX microtitre plate reader (Dynatech laboratories, UK).

3.4.4 Zinc Fingers and Homeoboxes Protein 1 (ZHX1)

Quantitative estimation of rat zinc fingers (ZHX1) was performed using a commercially available ELISA kit (CUSABIO©,Biotech Limited, China) according to the manufacturer's instructions. Briefly, 50µl of the sample was added to the wells of the microtitre plate where ZHX1 present were immobilized onto specific pre-coated antibodies in the wells of the plate. With alternating washing steps, biotin-conjugated ZHX1 specific antibodies followed by avidin conjugated horseradish peroxidase (HRP) were added to the wells. A volume of 90µlTMB substrate solution was then added and colour developed monitored. Then 50µl of the stop solution was added to stop the reaction after 5 minutes and optical density determined at 450nm and 630nm respectively using a microplate reader. The readings at 630nm were subtracted from the readings at 450nm to correct for optical imperfections in the plate.

3.4.5 Thiobarburic Acid Reactive Substances (TBARS) Assay

This test was performed using a commercially available Kit (QuantiChrom™, Gentaur Molecular Products, Kampenhout, Belgium). This assay is based on the reaction of malondialdehyde (MDA), a principle Thiobarburic Acid Reactive Substances (TBARS) with Thiobarburic acid (TBA) to form a pink chromogen attributable to an MDA-TBA_2 adduct. The colour intensity at 535nm is directly proportional to TBARS concentration in the sample. Briefly, 1.5mM of MDA standard solution was prepared in distilled water and serial dilutions made using the same diluents in the wells of the microtitre plate to final concentrations of 30, 18, 9 and 0µmol/l. Subsequently, a volume of 200µl of the TBA reagent was added to a volume of 200µl of each standard and sample and the mixture incubated at 100^{o}C for 60 minutes. The mixture was then cooled down to room temperature centrifuged and a 100µl of the same loaded in duplicate onto clear flat bottom 96 well plates and optical density determined at 535nm.

3.4.6 Determination of Alanine Aminotransferase (ALT)

A clinical biochemical analyzer (Humalyzer 2000, Wiesbaden, Germany) was used to analyze serum samples for ALT using commercial reagent kits (Human Diagnostics, Wiesbaden, Germany) according to the manufacturer's instructions. Briefly, take two clean and dry test tubes and label them B for blank and TS for test sample.Add 0.5 ml of the substrate to each tube and incubate for 3 minutes and add 0.1ml of distilled water to the blank and 0.1 ml of serum to the test sample tube and incubate both tubes at 37^{o}C for 30 minutes. Then add 0.5ml of DNPH reagent to all tubes, mix well and leave to stand for 20 minutes at room temperature. Finally add 5ml of 0.4N NaOH reagent. Mix well and allow it to stand at RT for 10 minutes.. Measure the absorbance of the test samples against the blank at 505nm and read the activity against a standard curve.

3.4.7 Determination of Aspartate Aminotransferase (AST)

A clinical biochemical analyzer (Humalyzer 2000, Wiesbaden, Germany) was used to analyze serum samples for AST using commercial reagent kits (Human Diagnostics, Wiesbaden, Germany) according to the manufacturer's instructions. Briefly, take two clean and dry test tubes and label them B for blank and TS for test sample. Add 0.5 ml of the substrate to each tube and incubate for 3 minutes and add 0.1ml of distilled water to the blank and 0.1 ml of serum to the test sample tube and incubate both tubes at 37^{o}C for 30 minutes. Then add 0.5ml of DNPH reagent to all tubes, mix well and allow to stand for 20 minutes at room temperature. Finally add 5ml of 0.4N NaOH reagent. Mix well and allow it to stand at RT for 10 minutes. Measure the absorbance of the test samples against the blank at 505nm and read the activity against a standard curve.

3.4.8 Determination of Alkaline Phosphatase(ALP)

A clinical biochemical analyzer (Humalyzer 2000, Wiesbaden, Germany) was used to analyze serum samples for ALP using commercial reagent kits (Human Diagnostics, Wiesbaden, Germany) according to the manufacturer's instructions. Briefly, take four clean and dry test tubes and label them B for blank, C control, S standard and TS for test sample. Add 0.1 ml of the substrate, 1.0ml of the buffer to each tube. Then add 1.05 ml of distilled water to the blank and 1.0ml to the rest of the tubes. Incubate for 3 minutes and add 0.1ml of distilled water to the blank and 0.1 ml of serum to the test sample tube and incubate both tubes at 37^{o}C for 3 minutes. Then add 0.05ml of the phenol standard reagent to the tube labeled S all and 0.05 to the tube labeled TS, mix well and allow standing for 15 minutes at 37^{0}C. Finally add 5ml of 1ml of the colour reagent to all the tubes and 0.05ml of the sample to the tube

labeled C. Mix well after each addition and measure the absorbance of the test samples, control, standard and the blank against distilled water at 510nm.

3.4.9 Determination of Total Protein Levels

A clinical biochemical analyzer (Humalyzer 2000, Wiesbaden, Germany) was used to analyze serum samples for total proteins using commercial reagent kits (Human Diagnostics, Wiesbaden, Germany) according to the manufacturer's instructions.Briefly. Take 9 tubes and label them as blank and 1-8. Then make dilutions of the protein (BSA) standards with concentrations of 1,2,4,6,8 10mg/200µl by transferring respective amount of BSA from the standard protein solution (50mg/ml) and adjust it to a total volume of 200µl by adding distilled water. Add 2m of biuret reagent to all the tubes including the blank and the unknown samples tubes. Mix well and let them stand at RT for 10 minutes. Read the wavelengths at 540nm and get the values of the unknown samples from the standard curve.

3.4.10 Determination of Serum Albumin Levels

A clinical biochemical analyzer (Humalyzer 2000, Wiesbaden, Germany) was used to analyze serum samples for albumin using commercial reagent kits (Human Diagnostics, Wiesbaden, Germany) according to the manufacturer's instructions. Briefly, three tubes were labeled as blank B, test T and standard S. Add 2ml of the bromocresol blue to each of the tubes. Finally add 10ul of the standard into the tube S, 10ml of double distilled water to B and 10ul of serum to T and incubate at 37^0C for 10 minutes. The absorbance of the standard and the sample was read against the blank at 546nm.

3.4.11 Determination of Cadmium Chloride in Tissues

Brain, liver, kidney, bone and testes tissues were oven dried and the dry matter determined, after which 0.15mg of the dry matter content was weighed and transferred into the microwave digestion vessel into which 6ml of nitric acid and 1ml of hydrogen peroxide were added . Samples were digested in a Anton Paar Multiwave 3000 microwave system equipped with the acid digestion rotor 48MF50 (Anton Paar GmbH, Anton-Paar-Str. 20, A-8054 Graz, Austria). After digestion, the digestates were transferred to 50ml polypropylene vials, diluted with deionized water in a 1:25 ratio and the levels of $CdCl_2$ determined using an Inductively Coupled Plasma-Optical Emission Spectroscopy ICP-OES ICP-9000 system (Shimadzu, Japan).

3.5 Evaluation of Brain, Liver, Testes and Kidney's Pathological Changes

3.5.1 Hematoxylin and Eosin (H and E) Staining

Following euthinization of the rats, brains, testes, kidney and liver tissues were removed and fixed in 10% formal saline, embedded in paraffin wax and the paraffin blocks sectioned at a thickness of 3 to 4µm. Subsequently, the sections were stained with H and E stain and examined histologically under the light microscope to evaluate the effects of the test chelating/antioxidant agents on the development of organ toxicity following $CdCl_2$ challenge. This was done by checking whether there were any pathological lesions that would be due to $CdCl_2$ toxicity.

3.5.2 Brain Immunohistochemistry

Immunoperoxidase staining of 5µm fixed cryostat sections of the cerebral cortex and the cerebellum were carried out with glial fibirally acidic protein (GFAP) antibody. Briefly, brain samples were picked from rats of each experimental group, fixed and cryoprotected as described previously (Zhu *et al.,* 2001; Sinha *et al.,* 2009). Coronal sections of 5 µm of the cerebral cortex were made using cryomicrotome (Micron HM 520; Labcon). The sections were then mounted on 3-aminopropyltriethoxysilane-coated slide. Immunoperoxidase staining for GFAP (Monoclonal, 1:400) was carried out with 3,3'-Diaminobenzidine (*DAB*) chromogen and avidin-biotin complex (*ABC*) kit as described previously (Otani *et al.,* 2009). This was then visualized under optical microscope (Leica LAS EZ, Switzerland) to evaluate the ameliorating effect by looking at reduction in astrocyte reaction

3.6 Data Analysis

Data was stored in work sheets in Ms Excel and printed copies were filed for reference. The statistical analyses were done using Prism Graph pad software version 5. Results were given as mean $\pm$ standard error of mean (SEM) with significance level set at $p<0.05$. One way analysis of variance (ANOVA) was used to test for differences in means of total protein, GSH, ALP, AST, ALT, ZHX1 and $CdCl_2$ levels. Histopathology and immunohistochemistry data was qualitatively analysed.

CHAPTER FOUR
RESULTS

4.1 Analysis of Tea

The HPLC analysis effectively separated the the tea catechins and caffeine based on their elution profiles. Figures 2 and 3 present standard chromatograms with catechin and caffeine profiles for black and green tea, respectively.

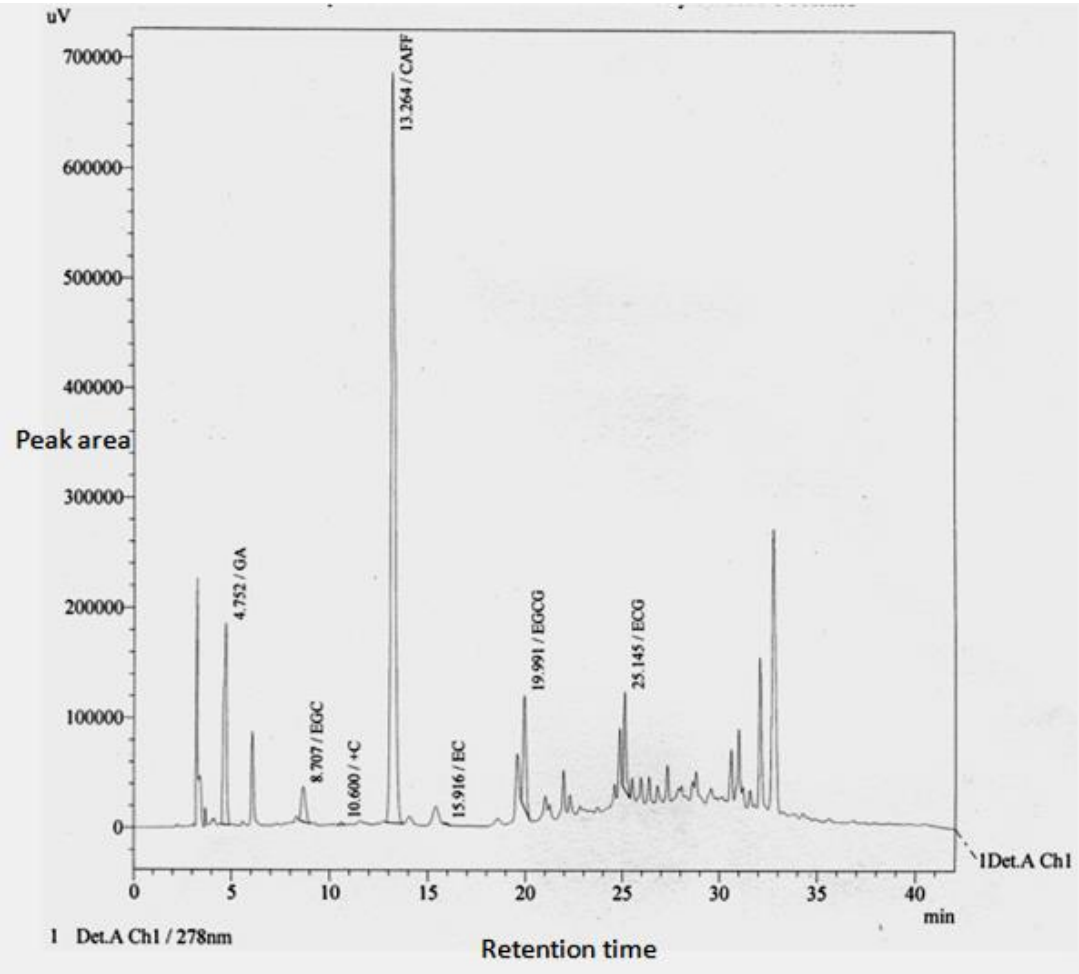

Figure 2: A chromatogram of Kenyan black tea variety TRFK 6/8 showing catechins elution time as follows, Gallic acid (GA), Epigallocatechin (EGC), Catechin (C), Caffein (CAFF), Epicatechin (EC), Epigallocatechin gallate (EGCG) and Epicatechin gallate (ECG)

KEY: X-axis = Retention time (min) Y-axis = Peak area

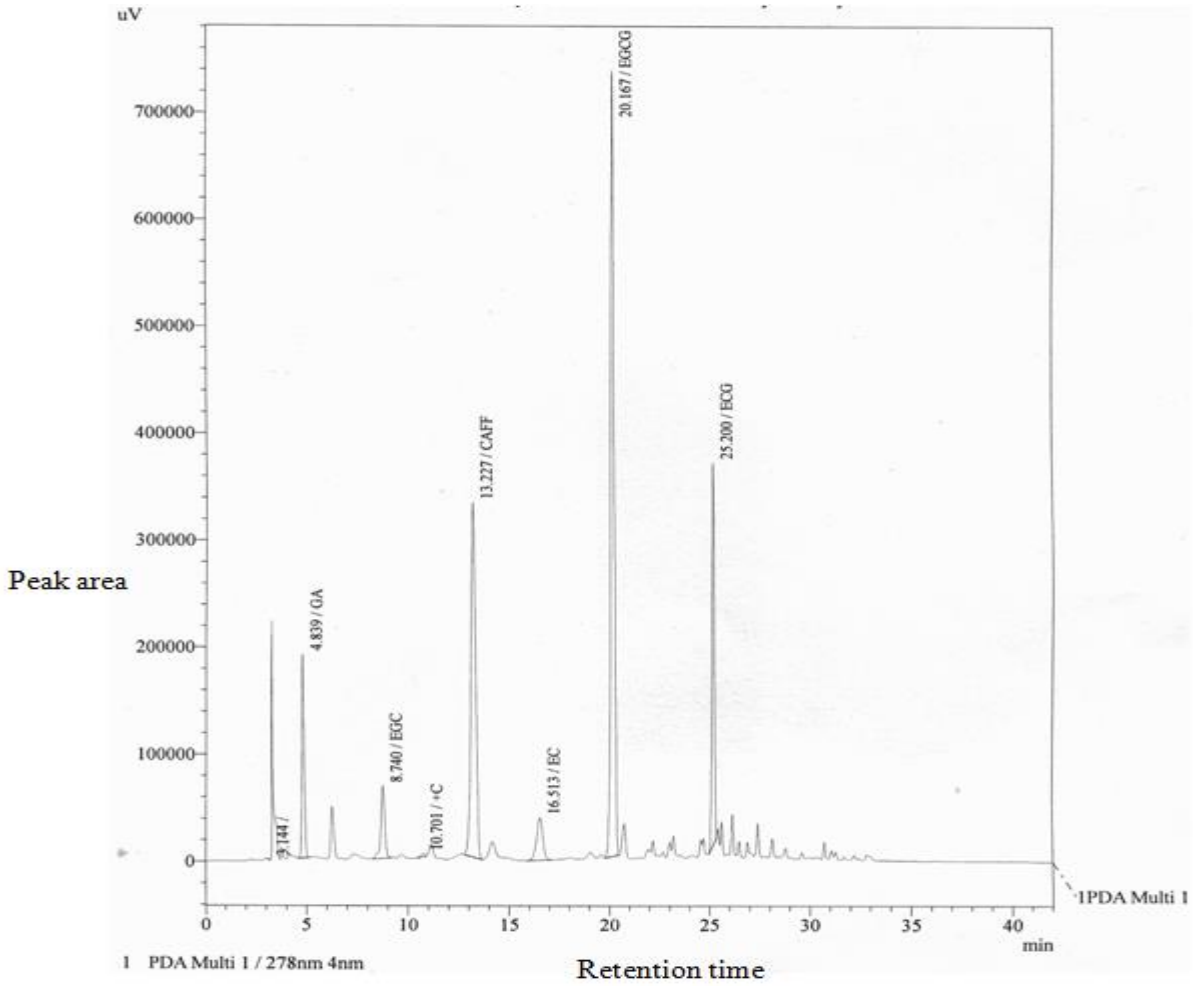

Figure 3 : A chromatogram of Kenyan green tea variety TRFK 6/8 showing catechins elution time as follows, Gallic acid (GA), Epigallocatechin (EGC), Catechin (C), Caffein (CAFF), Epicatechin (EC), Epigallocatechin gallate (EGCG) and Epicatechin gallate (ECG)

KEY: X-axis = Retention time (min) Y-axis = Peak area

Quantities of individual catechins (%) in the assayed black and green tea samples are presented in Table 1. The green tea samples had significantly ($P<0.05$) higher levels of EGCG, EGC, ECG and EC. There was no significant difference in C% between the two tea samples as shown in figure 2 and 3.

Table 2: Individual catechin (%) levels of black and green tea analyzed

Sample category (TRFK6/8)	EGCG%	EGC%	ECG%	EC%	C%
Black	[b]2.9±0.258	[b]1.7±0.175	[b]1.1±0.291	[b]0.5±0.161	[a]0.6±0.399
Green	[a]7.8±0.184	[a]4.3±0.597	[a]2.9±0.258	[a]1.5±0.245	[a]0.4±0.074

Means within a column with the same superscript letter(s) are not statistically different at p < 0.05.

Data on TP(%), AA(%), TF(%) and TR(%) is presented in Table 2. The green tea sample had significantly (p<0.05) higher TP (%) and AA(%) than black tea while TF(%) and TR(%) were significantly (p<0.05) higher in black than in green tea.

Table 3: Percent total polyphenols (TP), antioxidant capacity (AA), theaflavins and thearubigins of black and green tea analyzed

Sample category(TRFK6/8)	TP%	AA%	TF%	TR%
Black	[b]20.4±0.34	[b]77.6±0.83	[b] 2.6±0.04	[b]17.8±0.184
Green	[a]25.0±0.12	[a]88.7±0.47	[a]0.1±0.005	[a] 4.9±0.14

Means within a column with the same superscript letter(s) are not statistically different at p < 0.05.

The levels of cadmium and trace elements in the processed black and green tea samples are presented in Table 3. There were no significant (p<0.05) differences between the teas in the contents of Cd, Mg, Al, Mn, K, Cu, Fe, S, Zn and Ca.

Table 4: Levels of Cadmium and trace elements in tea

Sample category	Cd (ppb)	Mg (%)	Al (ppm)	Mn (%)	K (%)	Cu (ppm)	Fe (ppm)	S (%)	Zn (ppm)	Ca (%)
Black-tea (TRFK6/8)	0.63	0.55	4030	0.563	1.47	285	407	0.63	103	1.49
Green-tea (TRFK6/8)	0.61	0.52	3940	0.541	1.47	285	413	0.57	99.7	1.41

4.2 Effects of Tea and EDTA on ZHX1. TBARS and GSH levels in the Brain of Rats

4.2.1 Signs and Symptoms

Rats that were supplied with tea or EDTA recorded an increase in bwt throughout the experimental period. Additionally, rats that were intraperitoneally injected with EDTA showed signs of acute pain on injection. The pain was characterized by a prolonged sharp cry, followed by low pitch cries and at intervals that lasted at least for one hour. However, rats that were supplied with water only showed constant bwt with minor fluctuations throughout the experiment.

4.2.2 Percent Packed Cell Volume (PCV) Levels.

PCV levels of the experimental rats used in this study were determined prior to the start of the experiment to provide the baseline data as shown in table 4. There was a similar level of PCV at the start of the experiment that was followed by a steady decrease in PCV levels from 52.8±0.86 to 49.66±0.58 and 54.8±0.92 to 44.6±1.21 in rats that were supplied with black teaand EDTA, respectively. These levels were statistically different at $p<0.05$). There were no PCV changes in the rats that were supplied with green tea.

Table 5: Percent (%) PCV changes in rats supplied with black tea, green tea or EDTA compared with rats that were supplied with water only.

Weeks Post start of Experiment	Control	Black tea	Green tea	EDTA
0	57.4±0.74[a]	52.8±0.86 [a]	50.6±0.51[a]	54.8±0.92 [a]
1	57.14±2.76 [a]	52.26±0.78 [a]	50.82±1.31 [a]	54±0.77 [a]
2	57.36±2.72 [a]	50.2±0.58 [b]	49.28±0.77 [a]	50.7±0.86 [b]
3	57.2±1.98 [a]	50.2±0.64 [b]	49±0.32 [a]	50.6±0.4 [b]
4	57.0±2.43 [a]	48.2±0.86 [c]	49.2±0.37 [a]	48.2±1.2 [b]
5	56.0±2.84 [a]	46.8±0.77 [c]	47±0.54 [a]	45.52±1.08 [c]
6	57.4±2.76 [a]	49.66±0.58 [c]	49.04±0.31 [a]	44.6±1.21 [c]

Means within a column with the same superscript letter(s) are not statistically different at $p < 0.05$.

4.2.3 Body Weight of Rats

Rats that were treated with EDTA, green tea or black tea registered a significant ($p<0.05$) increase in bwt throughout the experiment (Figure 4). However, the bwt of the rats that were injected i.p with EDTA dropped at week 2 post administrations from 353.34±17.01g to 337.86±23.202g. There were no significant differences at weeks 3and 4, however there was a sharp increase from 336.20 ± 19.77 to 377 + 22.25g at week five post administration. Further, the bwt of the rats that received 200mg/kg body weight of black tea intra gavage dropped at week 4 post administrations from 320.88±22.61g to 280.52±25.26 and increased sharply at week 5 post administrations from 280.52±25.26g to 327.20±18.09g.

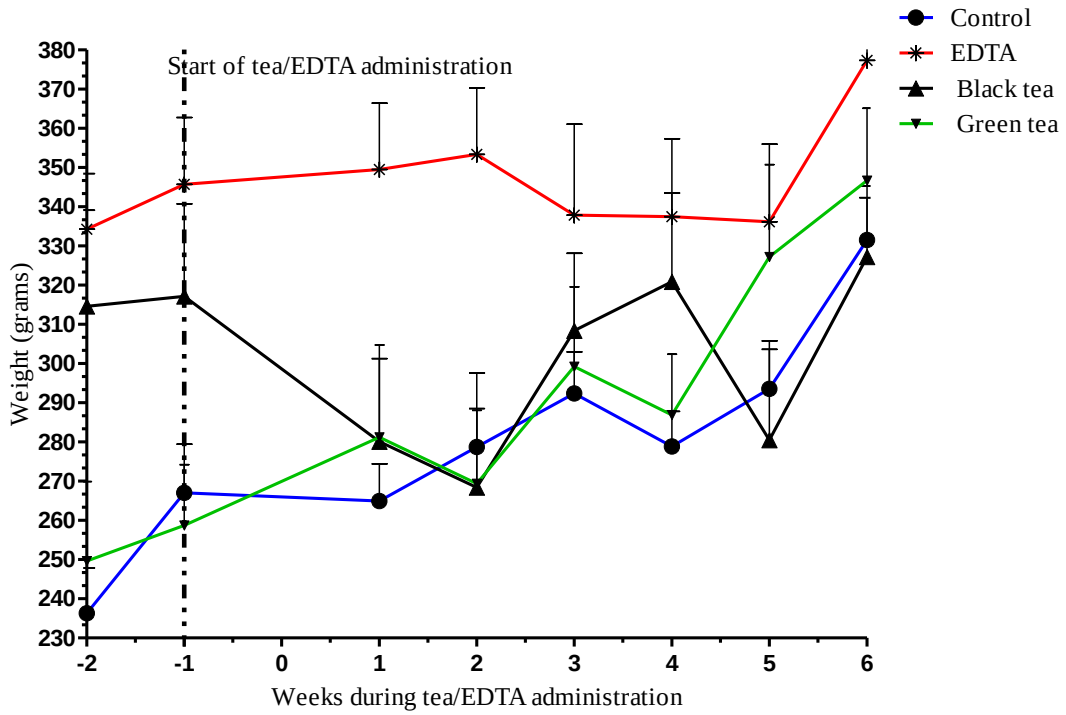

Figure 4: Changes in body weight of rats supplied with tea (black and green tea extracts) and EDTA

4.2.4 Brain Reduced Glutathione Levels

Levels of glutathione, the most common low molecular weight sulfhydryl-containing compound in the cells responsible for quenching free radicals, were determined in the brain of rats that were supplied with black tea, green tea or EDTA as shown in figure 6. The results showed that there was no significant ($p<0.05$) difference between rats that were supplied with either black tea, green tea when compared to the control animals that were supplied with water only. Secondly, there was no significant difference between the tea treated groups when

compared with groups that were treated with EDTA. GSH levels were however marginally higher in the tea treated animals.

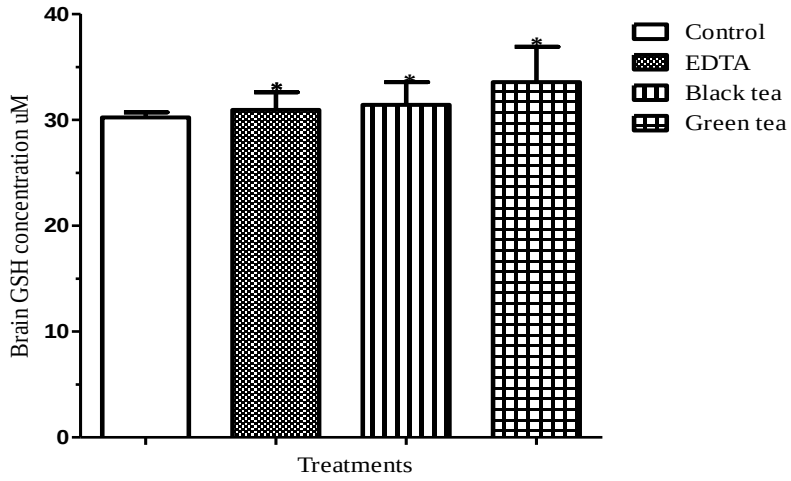

Figure 5: Effect of tea (black and green tea extracts) and EDTA on brain GSH levels in rats.

* denotes no significant (p<0.05) difference on GSH levels between rats that were supplied with either black tea, green tea and EDTA when compared to the control animals that were supplied with water only.

4.2.5 Brain Zinc Fingers and Homeoboxes Protein I Levels

Levels of Zinc-fingers and homeoboxes protein 1 transcription factors were determined in the brain of the rats (Figure 6). Results indicated that there was no significant (p<0.05) difference between the various treatment groups. At the same time, there was no significant (p<0.05) difference between the groups when compared with the control group though the tea treatments resulted in marginally lower levels of ZHX1. Though not significant, green tea registered lower ZHX1 levels than black tea when compared to the controls that received water only.

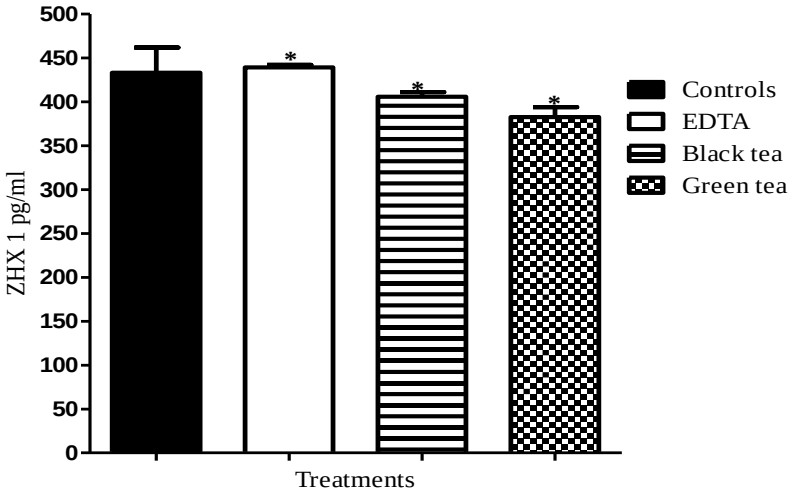

Figure 6: Effect of tea (black and green tea extracts) and EDTA on brain ZHX1 levels in rats.

* denotes no significant (p<0.05) difference on ZHX1 levels between rats that were supplied with either black tea, green tea and EDTA when compared to the control animals that were supplied with water only.

4.2.6 Brain Thiobarbaric Acid Assays Levels

Results of TBARS, the index of lipid peroxidation as determined by malonaldehyde (MDA) levels in the brain tissue of rats are shown in Figure 8. Results show no significant (p<0.05) difference in the MDA levels between the various treatment groups.

There was no significant (p<0.05) difference between the groups when compared with the control group.

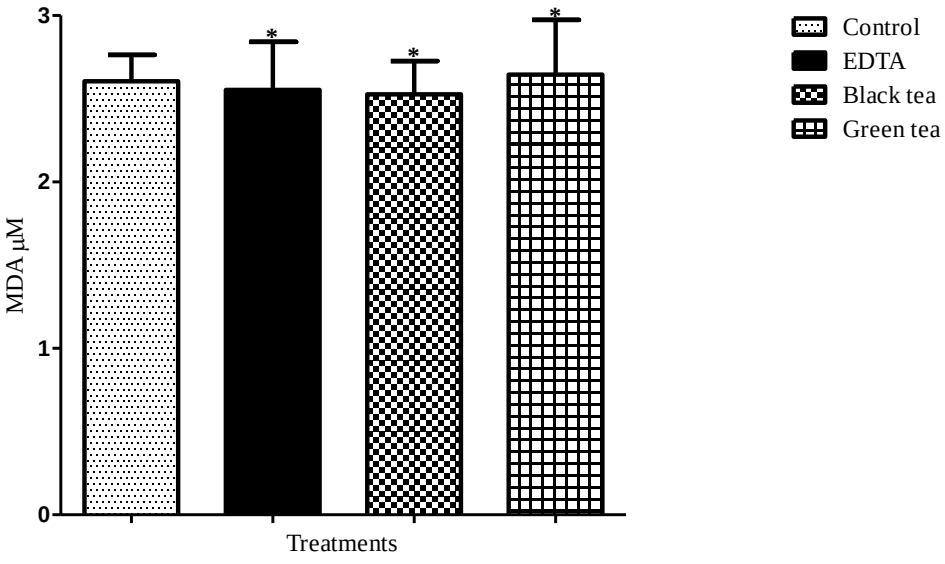

Figure 7:Effect of tea (black and green tea extracts) and EDTA on brain TBARS levels in rats.

* denotes no significant (p<0.05) difference on TBARS levels between rats that were supplied with either black tea, green tea and EDTA when compared to the control animals that were supplied with water only.

4.3 Effects of tea (Black Tea and Green Tea) aqueous Extracts Compared with EDTA Consumption on the liver healthy wistar rats

The present study showed that treatment of rats with tea extracts alone did not significantly (p<0.05) affect liver GSH, TBARS, ZHX1 tissue homogenates.

4.3.1 Liver Reduced Glutathione (GSH) Levels

Total glutathione concentrations were determined in the liver of the experimental rats as shown in Figure 9. No significant (p<0.05) differences were recorded between the various treatment groups. There was also no significant (p<0.05) difference between the groups when compared with the control group

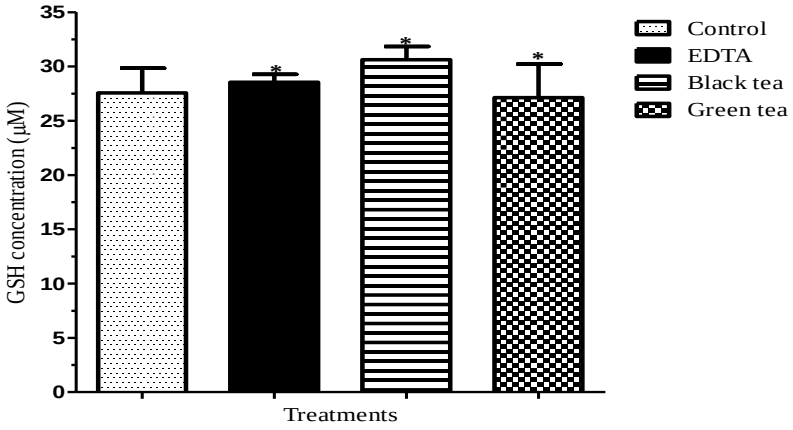

Figure 8: Effects of tea (black and green tea extracts) and EDTA on liver GSH levels in rats.

* denotes no significant (p<0.05) difference on liver GSH levels between rats that were supplied with either black tea, green tea and EDTA when compared to the control animals that were supplied with water only.

4.3.2 Liver Zinc Fingers and Homeoboxes Protein I (ZHX1) Levels

Levels of Zinc-fingers and homeoboxes protein 1 transcription factors were determined in the liver of the rats as shown in Figure 9. No significant (p<0.05) differences were recorded between the various treatment groups. In addition there was no significant (p<0.05) difference between the treatment groups when compared with the control group

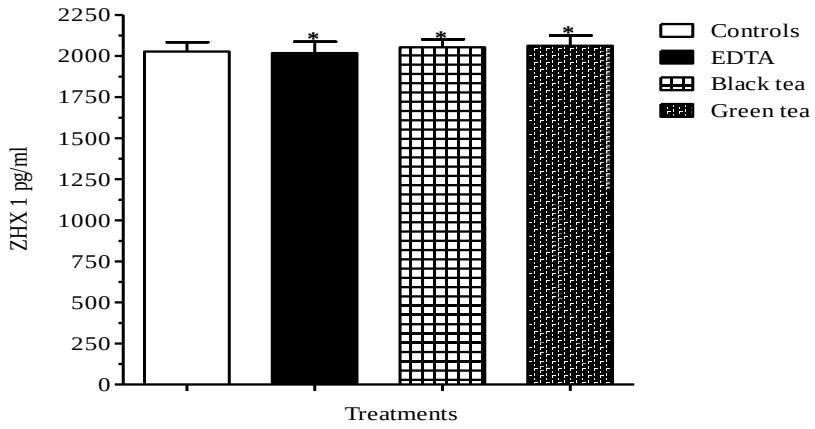

Figure 9: Effect of tea (black and green tea extracts) and EDTA on liver ZHX1 levels in rats.

* denotes no significant (p<0.05) difference on liver ZHX1 levels between rats that were supplied with either black tea, green tea and EDTA when compared to the control animals that were supplied with water only.

4.3.3 Liver Thiobarburic Acid Assays (TBARS) Levels

Data on liver thiobarburic acid assays is presented in Figure 10. Results indicate that there was no significant (p<0.05) difference in the MDA levels between rats that were supplied with either black tea or green tea when compared to the control animals that were supplied with water only. On the contrary, EDTA significantly (p<0.05) decreased liver MDA levels when compared to the control animals that received water only and animals that received either black tea or green tea.

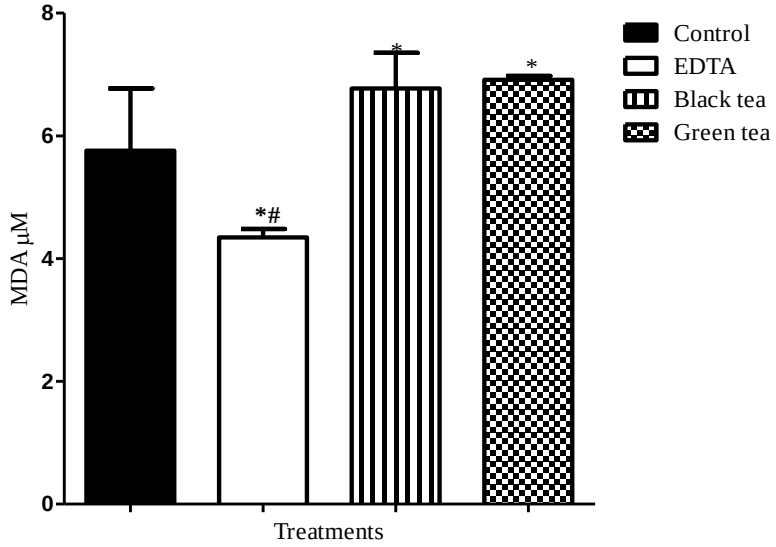

Figure 10: Effectof tea (black and green tea extracts) and EDTA on liver TBARS levels in rats.

* denotes no significant (p<0.05) difference on liver TBARS levels between rats that were supplied with either black tea, green tea when compared to the control animals that were supplied with water only.

*# denote significant p<0.05 difference on liver TBARS when compared to rats that were supplied with either green tea or black tea.

4.4 Liver Function Tests (LFTs)

LFTs were determined in the experimental rats as shown in the data presented in Figures 12-16. The aminotransferases and alkaline phosphatase assays, assessed the integrity of the hepatocytes and the total proteins and albumin levels evaluated the protein biosynthesis capacity of the liver

Alanine aminotransferase (ALT) was determined in serum of rats as shown in Figure 11. Results show no significant (p<0.05) differences between the various treatment groups. Similarly there was no significant (p<0.05) difference between the groups when compared with the control group

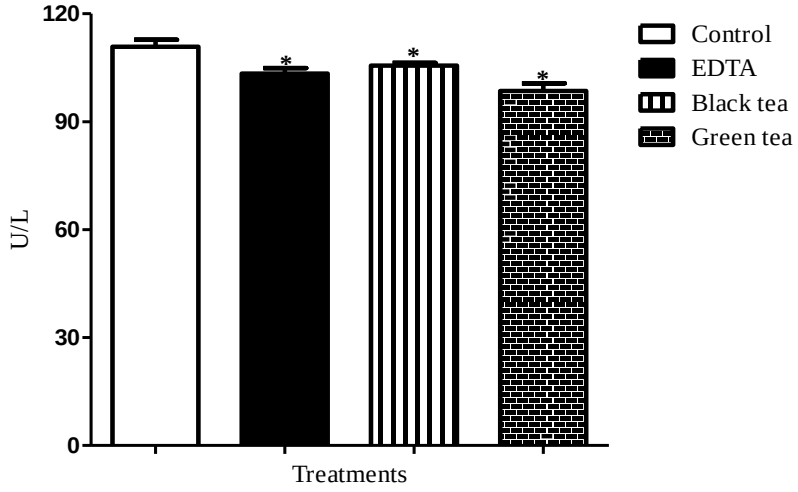

Figure11: Effectof tea (black and green tea extracts) and EDTA on serum ALT levels in rats.

* denotes no significant (p<0.05) difference on serum ALT levels between rats that were supplied with either black tea, green tea and EDTA when compared to the control rats that were supplied with water only.

Additionally, there was significant p<0.05 difference on serum AST levels of green tea, black tea and EDTA when compared to the control rats that received water only (Figure 13).

At the same time there were significant (p<0.05) difference between the groups. EDTA and black tea had lower AST levels when compared to green tea and the control rats.

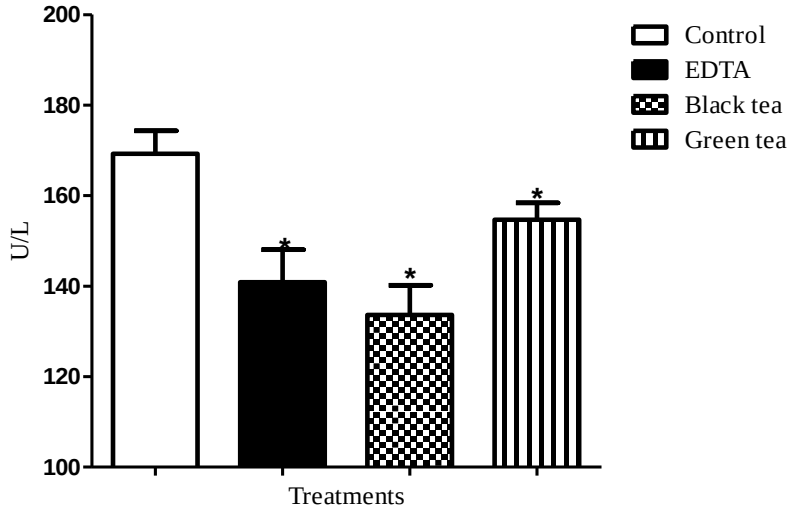

Figure 12: Effect of tea (black and green tea extracts) and EDTA on serum AST levels in rats.

*denotes significant p<0.05 difference on serum AST levels when compared to the control rats that received water only.

Likewise, results show significant (p<0.05) differences in the serum ALP levels between rats that were supplied with either black tea, green tea or EDTA when compared to those that received water only as shown in Figure 13. There was a significant (p<0.05) decrease in ALP levels in EDTA, black and green tea with green tea showing the greatest decline.

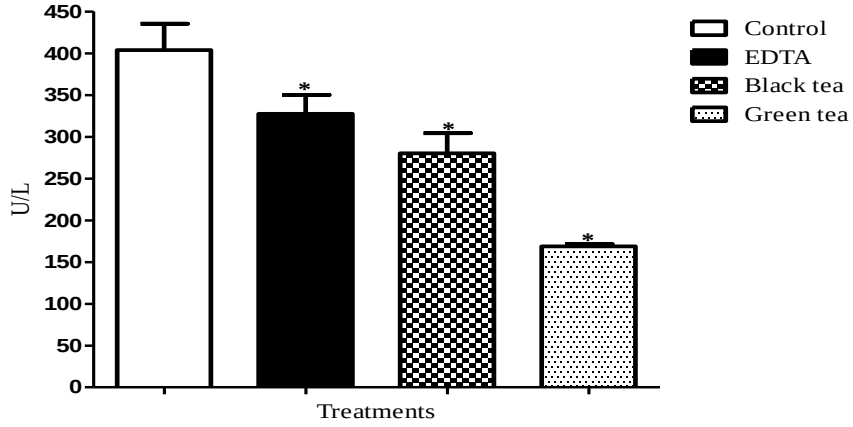

Figure 13: Effect of tea (black and green tea extracts) and EDTA on serum ALP levels in rats.

*denotes significantly (p<0.05) different serum ALP when compared to the control rats that received water only.

Moreover, the mean total protein levels in all treatment groups ranged from 7.4 g/dl to 7.6 g/dl. No significant (p<0.05) differences were recorded between black tea, green tea and EDTA groups. At the same time there was no significant (p<0.05) difference between the groups when compared with the control group.

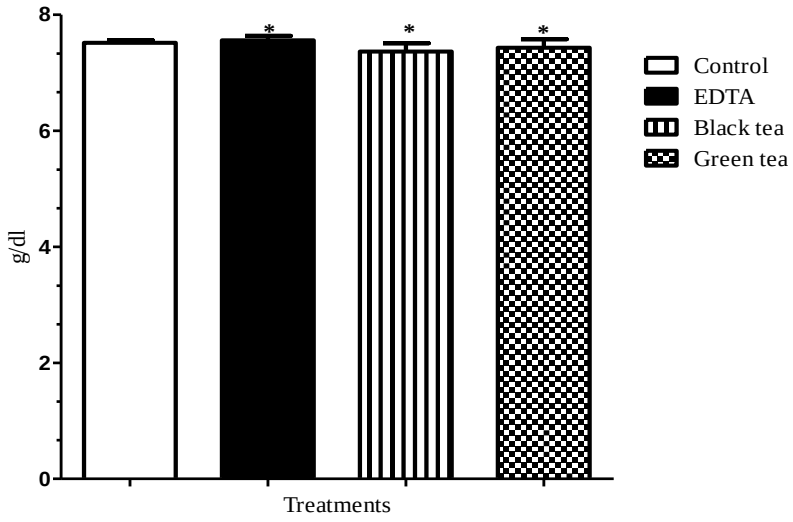

Figure 14: Effect of tea (black and green tea extracts) and EDTA on total protein levels in rats.

* denotes no significant (p<0.05) difference between the groups when compared with the control group

Finally, EDTA significantly (p<0.05) increased the albumin levels in serum of experimental rats when compared to animals supplied with either green tea or black tea and the control groups that were given regular drinking water (Figure 15). There was no significant (p<0.05) difference between black tea and tea groups. Results also showed no significant (p<0.05) difference between the two tea groups when compared with the control group

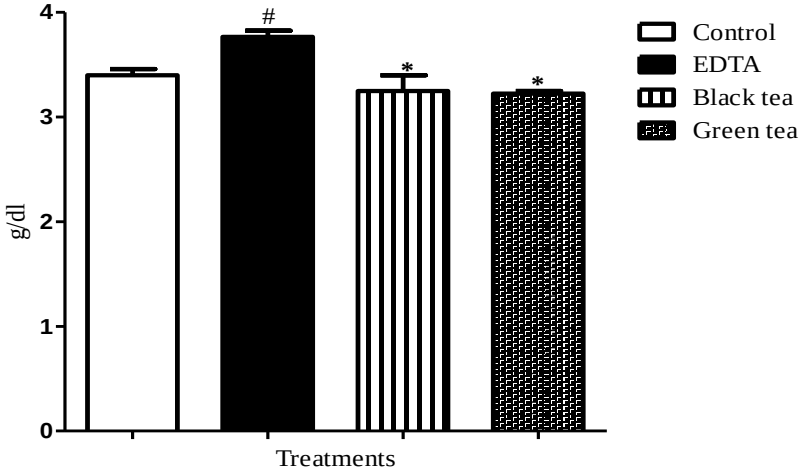

Figure 15: Effect of tea (black and green tea extracts) and EDTA on serum albumin levels in rats. *denotes no significant (p<0.05) difference between green tea and black tea when compared with the control group#denotes significantly (p<0.05) different albumin levels when compared to the control rats that received water only and black tea or green tea groups.

4.5 Protective Effects of Aqueous Black Tea and Green Tea Extracts Compared with EDTA during Cadmium Induced Toxicity in the Brain of Rats

4.5.1 Clinical Signs and Symptoms

Clinical signs and symptoms of the cadmium chloride treated rats included weight loss, scratching and hair loss at site of injection, diarrhea, generalized weakness, difficulty in walking and duck like gait posture. On termination of treatment mottling of the liver was evident. Additionally, there was testicular and kidney atrophy and hepatosplenomegally. All the animals survived to the end of the experiment and were sacrificed at day 42.

4.5.2 Percent (%) Packed cell Volume levels

Anemia which was characterized by a swift drop in PCV levels was observed after cadmium chloride administration. This occurrence advanced consistently up to the end of the experimental period (Figure 16). Following $CdCl_2$ administration, PCV levels dropped from 52.5±1.85%, 52.48±2.32% and 53.00±2.12% to 43.35±3.80%, 47.94±2.93% and 50.14±0.93% in $CdCl_2$ challenged, $CdCl_2$ challenged green tea and $CdCl_2$ EDTA groups, respectively. This was with exceptions of $CdCl_2$ black tea group that recorded an increase of PCV from 53.4±0.81% from 55.26±2.61%. There was a drop in PCV levels one week after experiment for all CdCl2 challenged groups with or without green or black tea. However, CdCl2 and EDTA

had a less marked decrease in PCV levels when compared with that of CdCl2 and green tea group which showed the greatest drop.

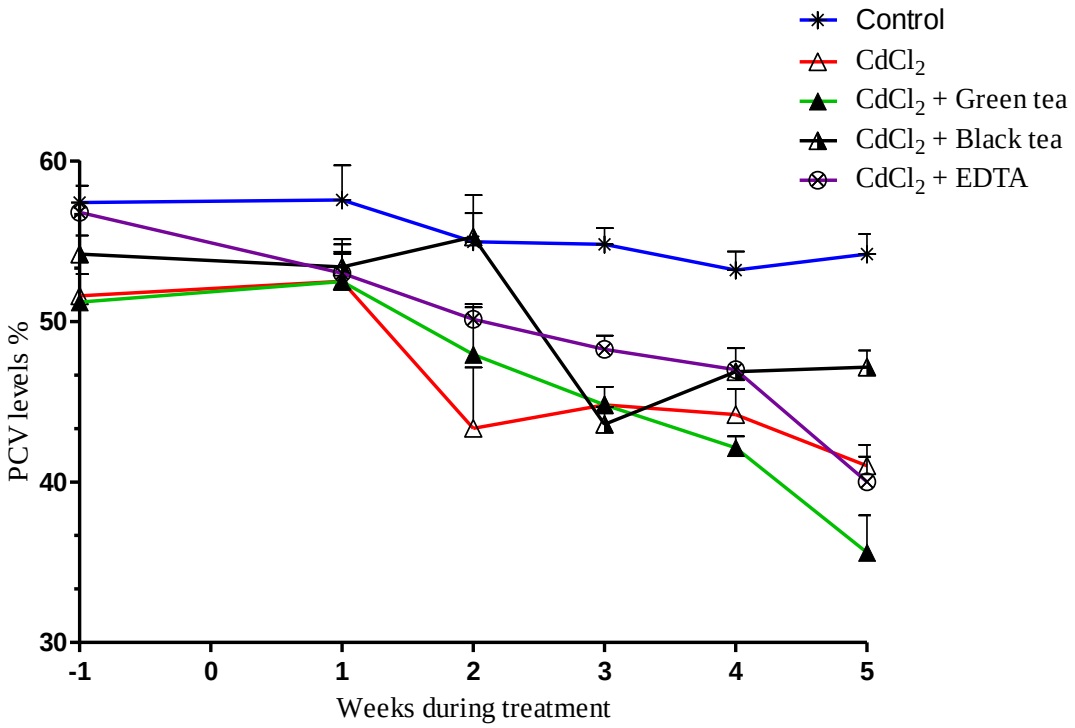

Figure 16: Percent PCV changes in rats given tea (black and green tea extracts) and EDTA and injected with$CdCl_2$ compared with control rats that were orally given water only.

4.5.3 Body Weight Levels

Control rats showed a continuous increase in body weight up to the end of the experimental period. On the other hand, rats challenged with $CdCl_2$ and water only recorded an increase in body weight but there was a decline in btwfrom 360.62±20.87 to 331.93±22.28g starting week four to the end of the experimental period. Rats challenged with $CdCl_2$ and supplemented with black tea recorded a decline in btw up to week two after which therewas an increase in week three and four. Thereafter, there was a steady decline in bwt from 341.7±10.47 to 307.56±8.79g beginning at week four to the end of the experimental period. Additionally, rats treated with $CdCl_2$ and supplemented with green tea recorded a decline in btw up to week two after which there was an increase in week three and four. Thereafter, there was a steady decline in bwt from 324.15±29.26 to 305.56±22.67 beginning at week four to the end of the experimental period. On the other hand, rats that were treated with $CdCl_2$ and supplemented with EDTA recorded an increase in bwt up to week three followed by a continuous decline to the end of experimentfrom 256.58±20.58 to 225.2±16.06g. Control rats recorded continuous increase in body weight from the onset of the experiment to the end.

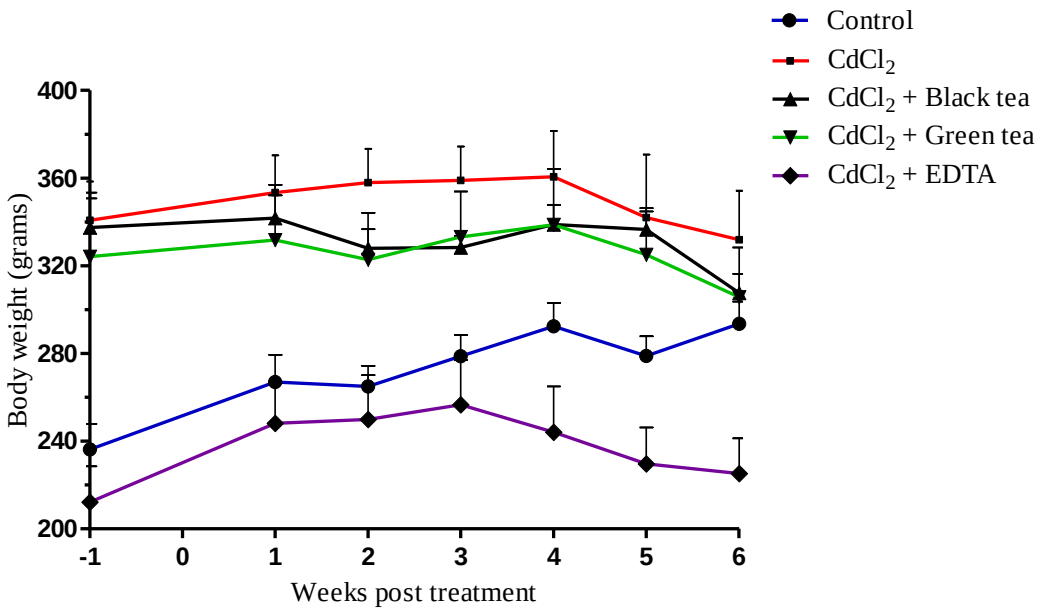

Figure 17: Changes in body weight of rats (Mean±SEM) treated with $CdCl_2$ and giventea (black and green tea extracts) and EDTAwith control rats that were orally given water only

4.6 Effects of Aqueous Black Tea and Green Tea Extracts Compared with EDTA during Cadmium Induced Toxicity in the Brain of Rats

Brain of rats challenged with $CdCl_2$alone recorded significantly higher ($p<0.05$) brain GSH levels when compared to animals supplied with water only, signifying a defensive response in the oxidative challenge posed by $CdCl_2$ administration (Figure 18). Rats challenged with $CdCl_2$ and supplemented with either black tea or green tea or the conventional chelating agent EDTA recorded significantly ($p<0.05$) reduced brain GSH levels when compared to animals challenged with $CdCl_2$ and supplied with water only. Notably, there were significant differences ($p<0.05$) in GSH levels between the conventional chelating agent, EDTA, and the experimental animals supplied with either green or black tea with the tea treatment showing lower levels of GSH. Green tea, black tea and EDTA showed an increase in GSH when compared with the control group; however, the increase was lower when compared with the rats that were challenged with $CdCl_2$alone.

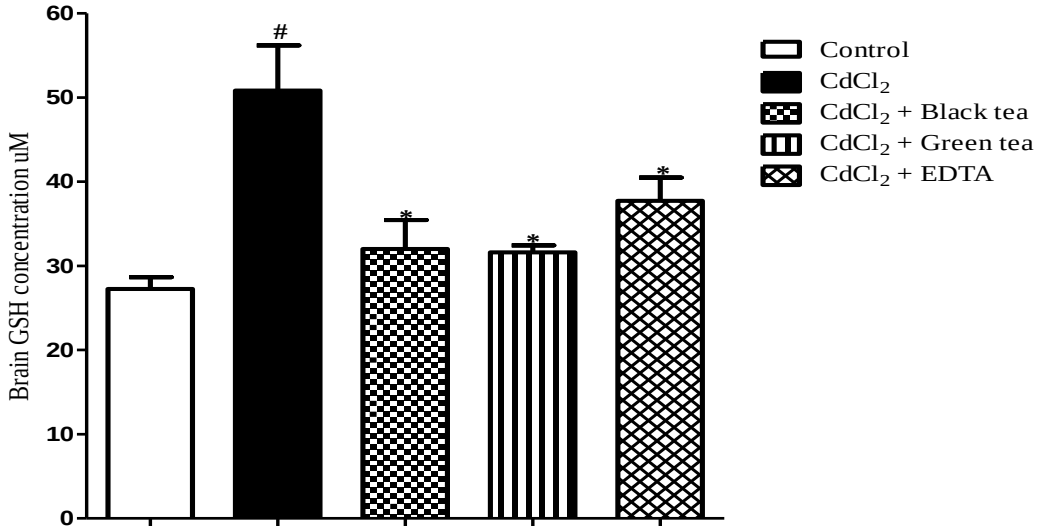

Figure 18: Effect of $CdCl_2$ exposure with black tea, green tea and EDTA on brain GSH levels in rats.#statistically significant versus controls, *statistically significant versus $CdCl_2$ group.

Additionally, levels of nuclear homodimeric transcriptional repressors were determined in rat brains as shown in Figure 20. Brain ZHX1 reduced significantly (p<0.05) in rats that were challenged with $CdCl_2$ and water only when compared to the control group supplied with regular drinking water only. Animals challenged with $CdCl_2$ and supplemented with green tea and EDTArecorded significantly (p<0.05) higher levels of brain ZHX1 when compared to animals challenged with $CdCl_2$ and supplied with water only. Moreover, $CdCl_2$ challenged animals supplemented with green tea, compared very closely with those supplemented with EDTA; a conventional synthetic chelating agent. Rats treated with black tea showed a slight decrease of ZHX1 levels when compared to the control group that received water only.

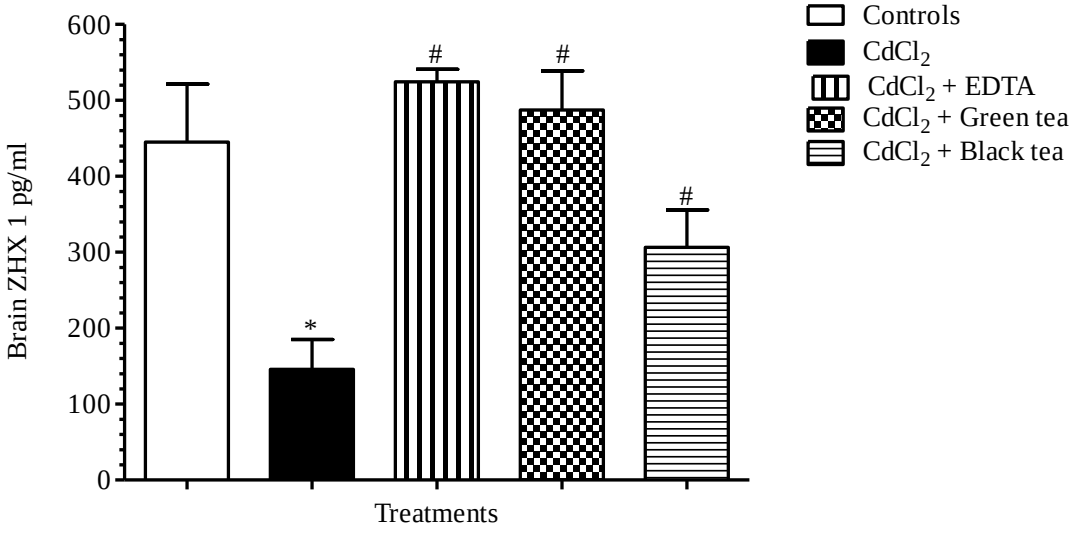

Figure 19: Effect of $CdCl_2$ exposure with black tea, green tea and EDTA treatment on brain ZHX1 levels in rats.*denotes a statistically significant (p<0.05) decline of ZHX1 levels when compared to green tea, black tea and EDTA groups. #shows that green tea, black tea and EDTA significantly (p<0.05) increased of ZHX1levels in the brain of rats that were challenged with CdCl2 when compared to rats challenged with $CdCl_2$ alone.

Furthermore, rats challenged with $CdCl_2$ recorded significantly higher (p<0.05) brain MDA levels when compared to control animals supplied with water only. Remarkably, animals challenged with $CdCl_2$ and supplemented with either black or green tea recorded significantly (p<0.05) lower brain MDA levels when compared to animals challenged with $CdCl_2$ and supplied water only. Both black tea and green tea were more potent than the conventional chelating agent EDTA in lowering MDA levels by 27.33% and 20.37%, respectively, when compared to 3.74% for EDTA as shown in Figure 20.

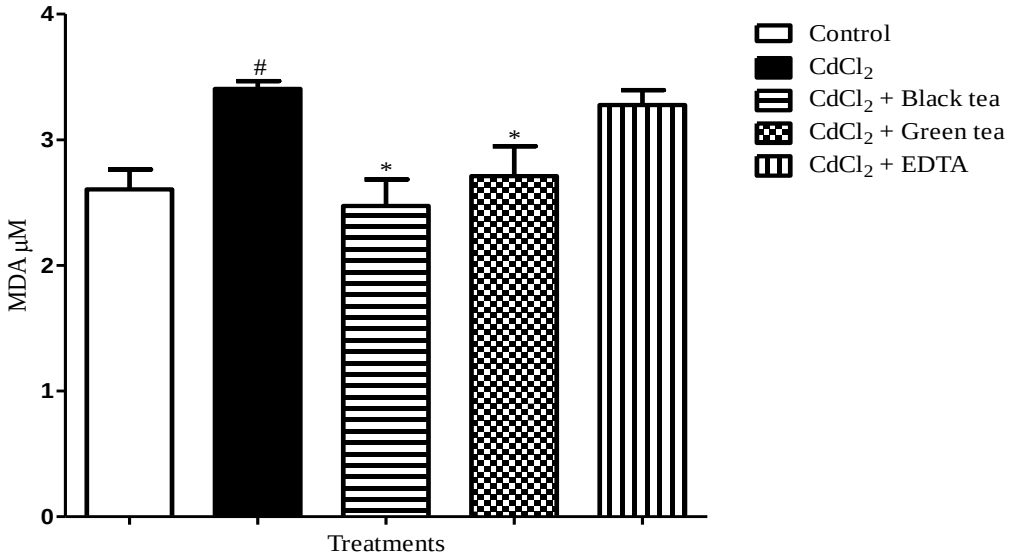

Figure 20: Effect of $CdCl_2$ exposure with black tea, green tea and EDTA treatment on brain MDA levels in rats.*indicates that both black and green tea are significantly (p<0.05) different when compared to $CdCl_2$ only challenged rats. #denotes that increase in MDAis statistically significant (p<0.05) in rats treated with $CdCl_2$ only when compared to control rats.

4.7 Effects of Aqueous Black Tea and Green Tea Extracts Compared with EDTA during Cadmium Induced Toxicity in the Liver of Rats

Levels of GSH, the primary protective mechanism against free radicals in the cells were determined in the liver of rats challenged with $CdCl_2$ and supplemented with either black tea or green tea (Figure 21). Results indicate that rats challenged with $CdCl_2$ alone recorded

significantly higher (p<0.05) liver GSH levels with a mean of 358.947 µMwhen compared to rats supplied with water only which had a mean of 96.783µM. Markedly, animals challenged with cadmium and supplemented with either black tea or green tea or EDTA recorded significantly (p<0.05) lower liver GSH levels of 83.430 µM, 66.231 µM and 77.501 respectively when compared to animals challenged with $CdCl_2$ only, implying a lower oxidative stress burden. No significant differences (p<0.05) were recorded between black tea, green tea treatment groups and EDTA.

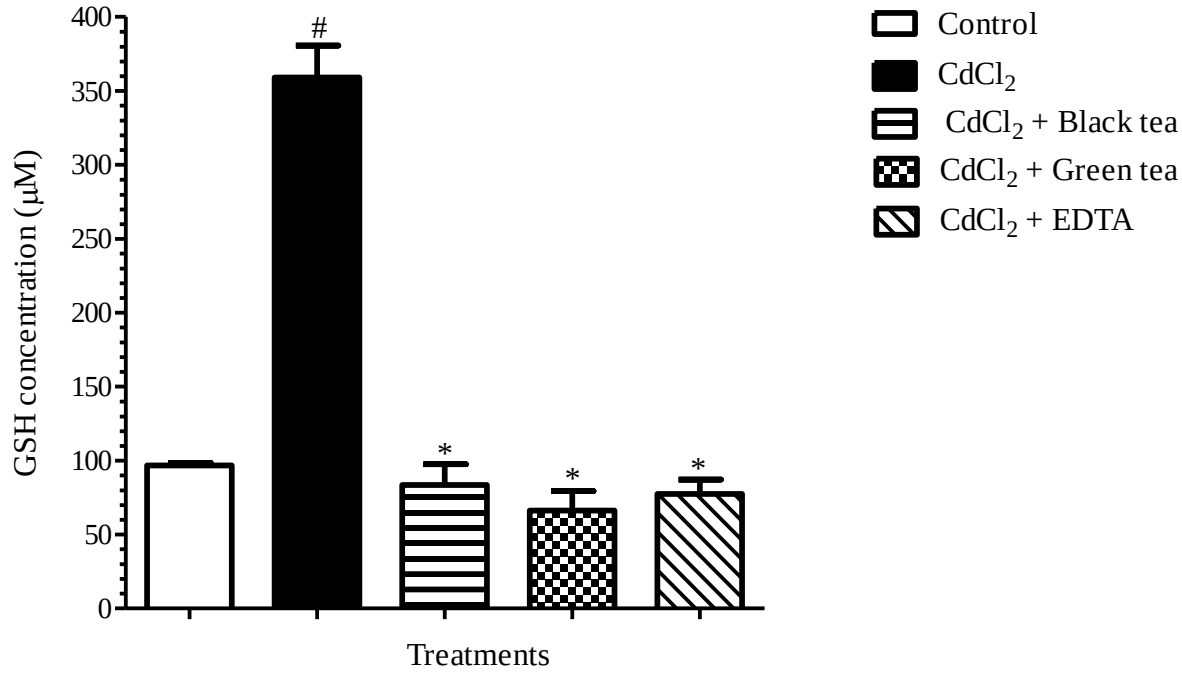

Figure 21: Effect of $CdCl_2$ exposure with black tea, green tea and EDTA treatment on liver GSH levels in rats.#significant (p<0.05) increase in liver GSH levels in $CdCl_2$only treated rats when compared to the controls. * significant (p<0.05) decrease in liver GSH levels in $CdCl_2$ plus either green tea, black tea and EDTA treated groups when compared with $CdCl_2$ only treated rats.

Moreover, data on the levels of zinc-fingers and homeoboxes protein 1 transcription factors that were determined in the liver of the rats treated with $CdCl_2$ and supplemented with black tea, green tea or EDTA is presented in Figure 22. Rats challenged with $CdCl_2$ and supplied with water only recorded significantly lower (p<0.05) liver ZHX1 levels when compared to animals supplied with water only. Remarkably, animals challenged with $CdCl_2$

and supplemented with either black tea or green tea recorded significantly (p<0.05) higher liver ZHX1 levels when compared to animals challenged with $CdCl_2$ alone.

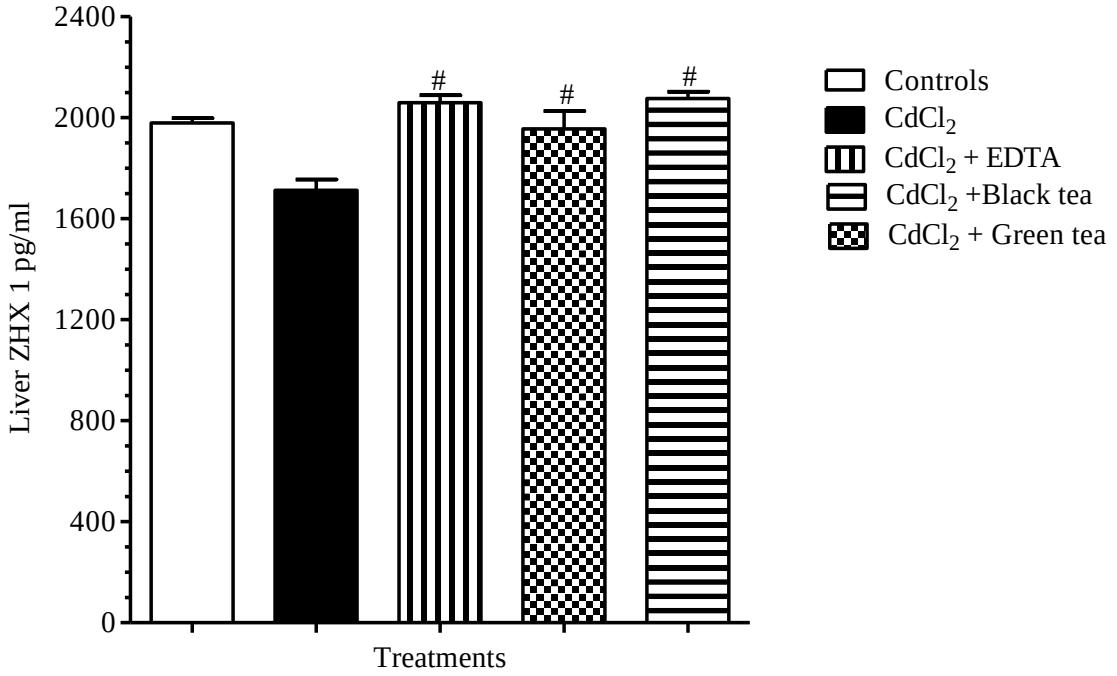

Figure 22: Effect of $CdCl_2$ exposure with black tea, green tea and EDTA treatment on liver ZHX1 levels in rats.#Green tea, black tea and EDTA significantly p<0.05 increased ZHX1 levels when compared to $CdCl_2$ challenged rats.

Likewise, thiobarburic acid assay which is a marker of lipid peroxidation (Tsai *et al*., 2015) was carried out in the liver tissue of rats challenged with $CdCl_2$ and supplemented with black tea, green tea and EDTA. Results obtained are presented in Figure 23 showed that $CdCl_2$ significantly (p<0.05) increased MDA levels in the liver of rats from an initial value of 5.76±1.01µM to 13.30±0.90µM when compared to the control animals supplied water only. Remarkably, green tea, black tea and EDTA groups significantly (p<0.05) lowered liver MDA levels from the initial 13.30±0.09µM recorded in untreated controls to 11.67±0.23µM, 10.24±0.63µM, and 9.360±0.42uM respectively. Most importantly, these results show that both aerated (black) and unaerated (green) tea, were as effective as EDTAwhich is a synthetic chelating agent.

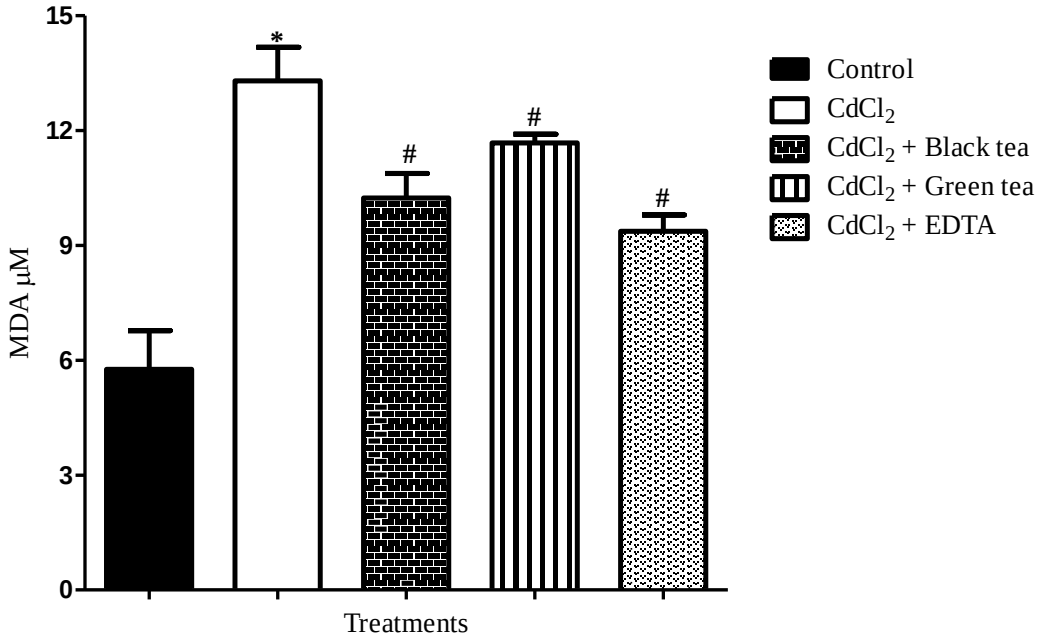

Figure 23: Effect of $CdCl_2$ exposure with black tea, green tea and EDTA treatment on liver MDA levels in rats.

#denotes that both black, green tea and EDTA are significantly (p<0.05) decreased liver MDA levels when compared to $CdCl_2$ challenged rats. *denotes that increase in MDA levels is statistically significant (p<0.05) when compared to control rats.

4.8 Liver Function Tests

As a measure of hepatocellular injury, ALT was determined in serum of rats challenged with $CdCl_2$ followed by black tea, green tea or EDTA treatment. ALT levels increased significantly (p<0.05) in $CdCl_2$ groups when compared to control groups on water only (Figure 24). Remarkably, animals challenged with $CdCl_2$ and treated with either black, green tea or EDTArecorded significantly (p<0.05) lower serum ALT levels when compared to animals challenged with $CdCl_2$ alone. Moreover, ALT levels in rats supplemented with either black or green tea had lower serum ALT levels comparable to control rats supplied water only. There was no significant differences (p<0.05) rats treated with either green tea or black tea when compared with EDTA; a conventional chelating agent.

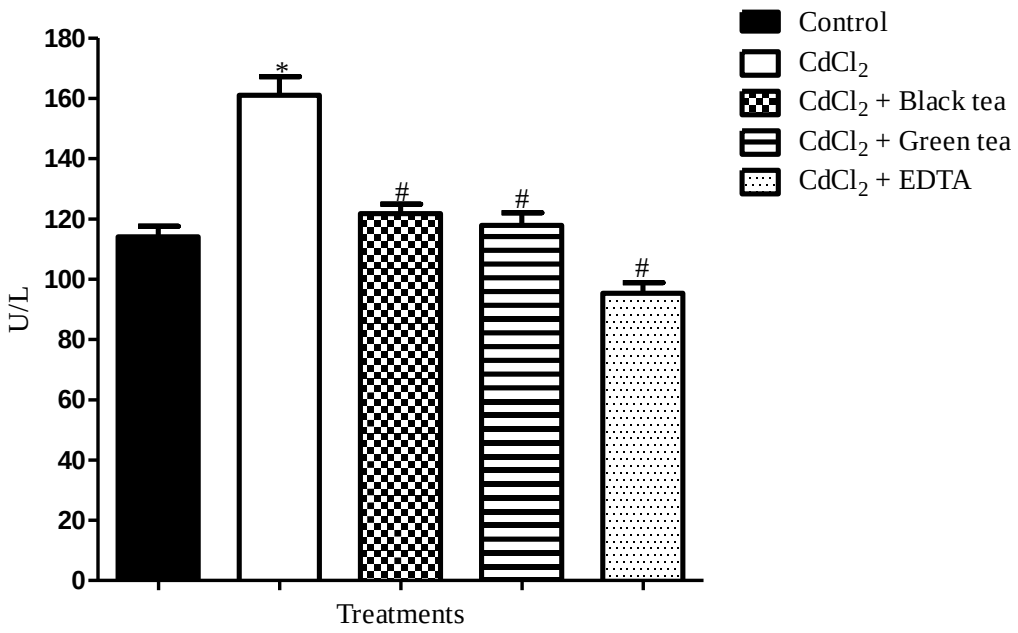

Figure 24: Effect of $CdCl_2$ exposure along with tea (aqueous black tea or green tea) and EDTA treatment on serum ALT levels.# denotes that green/black tea and EDTA significantly (p<0.05) decreased $CdCl_2$ induced ALT increase in rats. *denotes that $CdCl_2$ caused a significant (p<0.05) increase in ALT levels when compared to control rats.

Additionally, AST was determined in serum of rats to establish hepatocellular injury.$CdCl_2$ treatment resulted in significantly (p<0.05) higher AST levels when compared to control rats that received water only (Figure 25). On the other hand, rats challenged with $CdCl_2$ and supplemented with either black tea or green tea recorded significantly (p<0.05) lower AST levels when compared to animals challenged with $CdCl_2$ only. There were no significant differences (p<0.05) in AST levelsbetween green tea, black tea and EDTA treatment. Tea and EDTA treated groups showed significantly (p<0.05) increased AST levels when compared with the control groups.

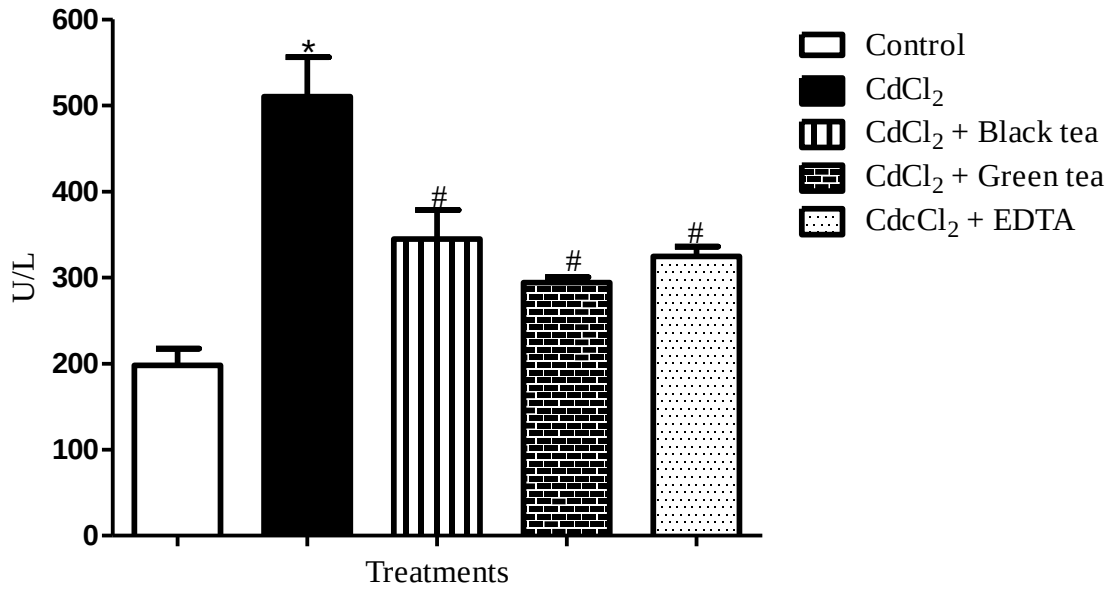

Figure 25: Effect of $CdCl_2$ exposure along with tea (aqueous black tea or green tea) and EDTA treatment on liver AST levels in rats.# denotes that green/black tea and EDTA significantly (p<0.05) inhibited $CdCl_2$ induced AST increase in rats. * denotes that increase in AST is statistically significant (p<0.05) when compared to control rats.

Additionally, ALP levels increased significantly (p<0.05) in rats challenged with $CdCl_2$ when compared to control rats that received water only (figure 26). Animals challenged with $CdCl_2$ and supplemented with tea (black tea or green tea) recorded significantly (p<0.05) lower ALP levels when compared to untreated $CdCl_2$ groups. Green tea and black tea's potency compared closely with that of the conventional chelating agent, EDTA, which also significantly (p<0.05) inhibited the $CdCl_2$ induced increases in ALP.

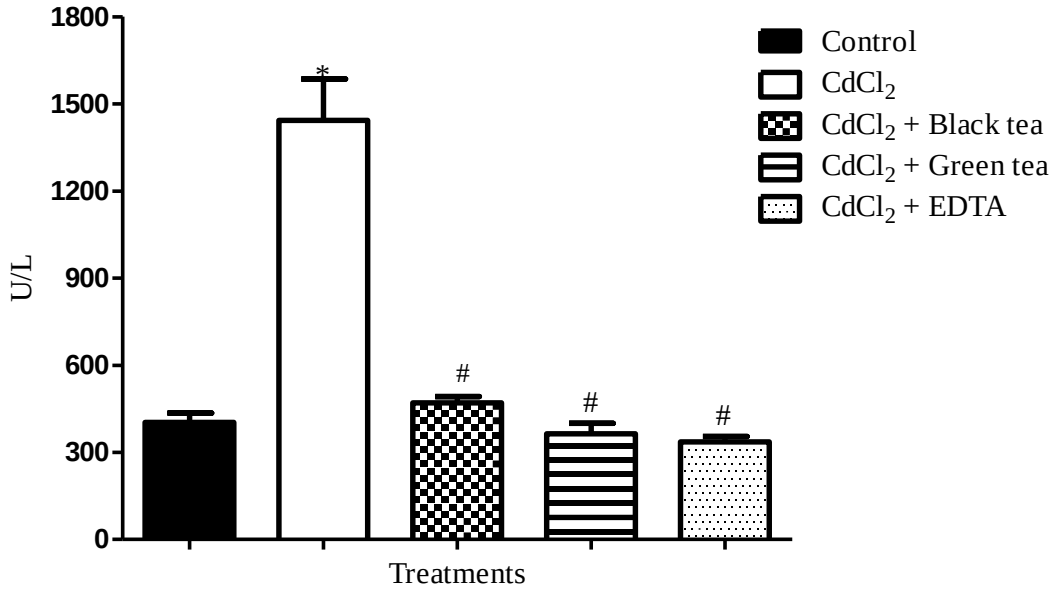

Figure 26: Effect of $CdCl_2$ exposure along with tea (aqueous black tea or green tea) and EDTA treatment on liver ALP levels in rats.#denotes that green/black tea and EDTA significantly (p<0.05) inhibited $CdCl_2$ induced AST increase in rats. *denotes that increase in ALPis statistically significant (p<0.05) when compared to control rats

Moreover, total proteins were determined in serum to assess the effect of $CdCl_2$ on the biosynthetic capacity of the liver. $CdCl_2$ treatment impaired liver biosynthetic capacity in rats as witnessed by the significantly (p<0.05) reduced the levels of total protein in serum (Figure 28). Both black and green tea were protective against $CdCl_2$ induced decreases in total proteins, with animals in these groups recording significantly (p<0.05) higher total proteins levels when compared to their untreated counterparts. Hepatoprotective effects signified by the increased total protein levels were comparable between the tea treatment groups and the conventional chelating agent, EDTA.

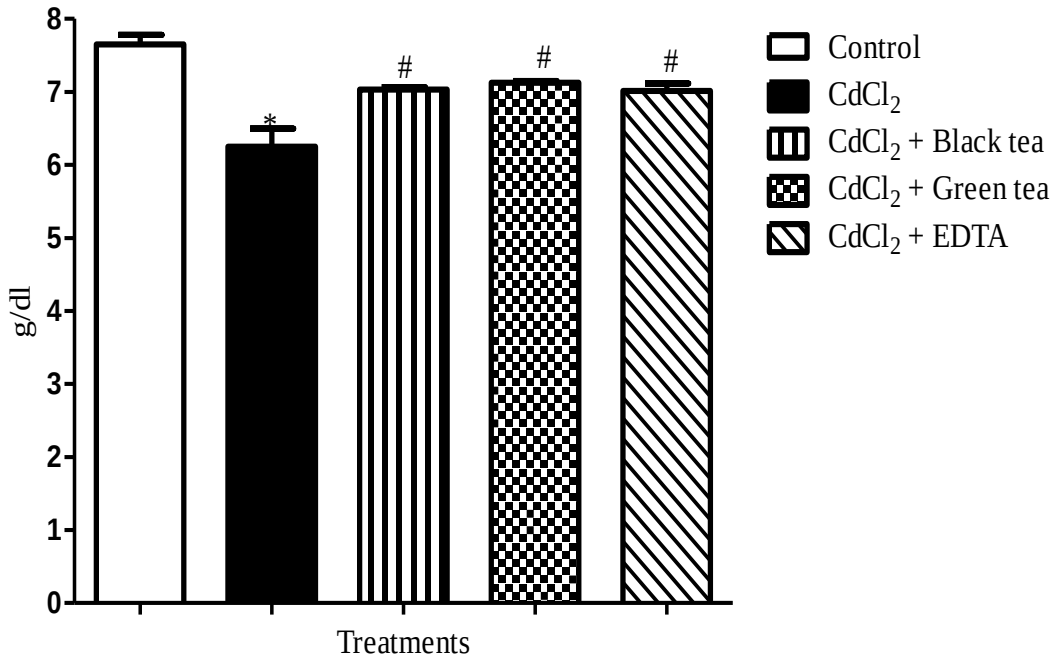

Figure 27: Effect of $CdCl_2$ exposure with or without chelating antioxidants intervention on protein levels in rats. #denotes that green/black tea and EDTA significantly (p<0.05) inhibited $CdCl_2$ induced protein decrease in rats. *denotes that decrease in proteinis statistically significant (p<0.05) when compared to control rats.

Likewise, to further characterize the $CdCl_2$ induced effects on total protein synthesis, levels of albumin, the most abundant plasma protein synthesized by the liver, were determined in the serum of experimental rats. $CdCl_2$ treatment significantly (p<0.05) reduced serum albumin levels in the experimental rats (Figure 28). Similarly, both black and green tea protected against the $CdCl_2$ induced decreases in serum albumin levels (p<0.05), with their effects being comparable to that recorded for EDTA which also significantly (p<0.05) inhibited the $CdCl_2$ induced decreases in serum albumin.

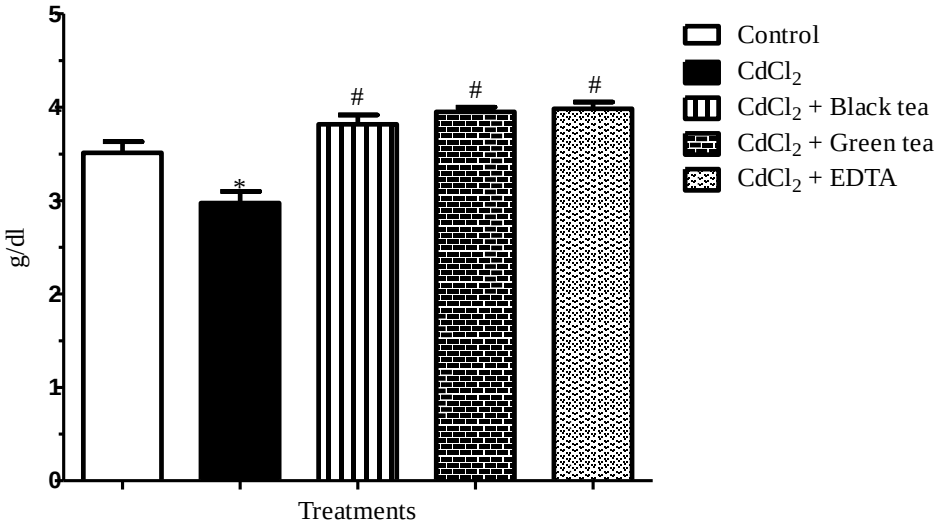

Figure 28: Effect of $CdCl_2$ exposure along with tea (aqueous black tea or green tea) and EDTA treatment on albumin levels in rats.#denotes that green/black tea and EDTA significantly (p<0.05) inhibited $CdCl_2$ induced albumin decrease in rats. *denotes that decrease in albumin decreaseis statistically significant (p<0.05) when compared to control rats.

4.9 $CdCl_2$ levels in Various Body Tissues

Levels of $CdCl_2$ in the kidney of rats that were challenged with $CdCl_2$ and water only increased significantly (p<0.05) when compared to the control animals that were given water only. Rats that were challenged with $CdCl_2$ and supplemented with green tea or black tea recorded a reduction of $CdCl_2$ levels in the kidney from 672.5±93.5ppb to 558.5±16.5ppb and 540.5±4.5ppb, respectively when compared with animals that were treated with $CdCl_2$ and water only. Both black and green teas were more potent and better protective agents than EDTA, the conventional chelating agent which recorded a reduction of 616±98ppb as shown in Figure 29.

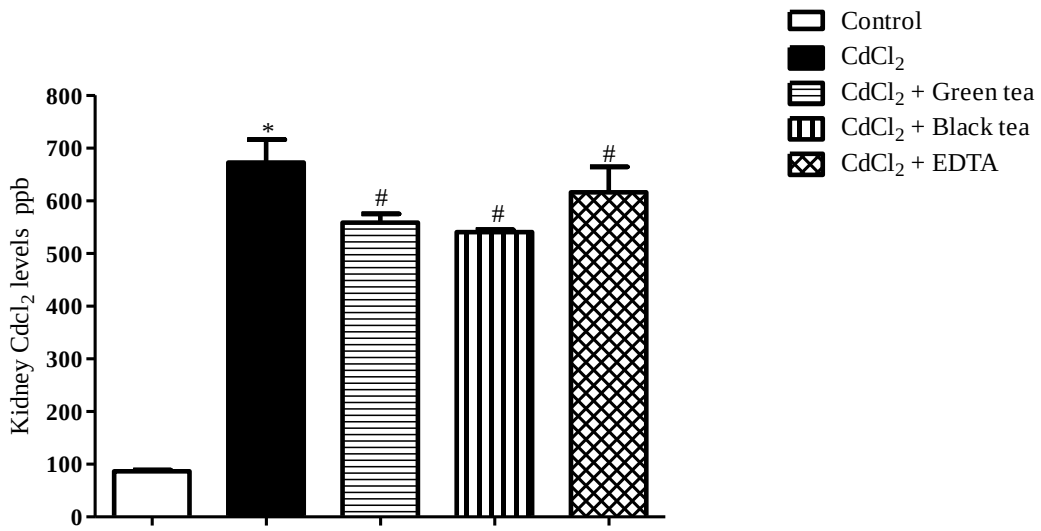

Figure 29: Effects of tea and EDTA on Levels of $CdCl_2$ in the kidney of rats.

#denotes that green/black tea and EDTA significantly (p<0.05) reduced $CdCl_2$ levels in the kidney of rats. *denotes that increase in $CdCl_2$ is statistically significant (p<0.05) when compared to control rats.

Furthermore, levels of $CdCl_2$ in the liver of rats that were challenged with $CdCl_2$ and water only increased significantly (p<0.05) from 10.33±0.33 to 65.33±1.856 ppb when compared to the control animals that were given water only. Rats that were challenged with $CdCl_2$ and supplemented with green tea or black tea recorded a noticeable reduction of $CdCl_2$ levels in the liver from 65.33±1.856 ppb to 53.5±0.5 and 49.67±6.3ppb, respectively, when compared with animals that were treated with $CdCl_2$ and water only. Both black and green tea were more potent and better in reducing the amount/levels of $CdCl_2$ than EDTA, the conventional synthetic chelating agent which recorded a reduction of 59.5±1.5 as shown in Figure 30.

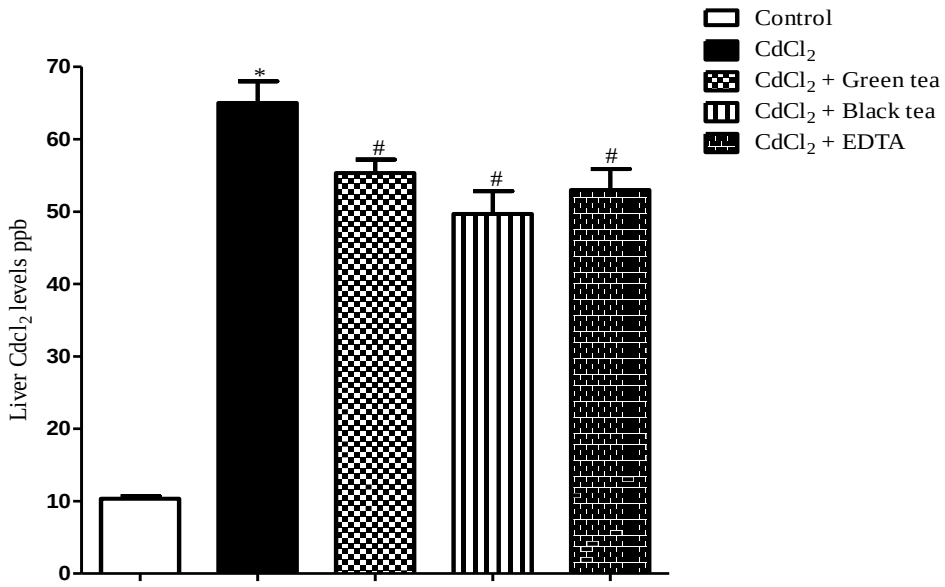

Figure 30: Effects of tea and EDTA on Levels of $CdCl_2$ in the liver of rats

#denotes that green/black tea and EDTA significantly ($p<0.05$) reduced $CdCl_2$ levels in the liver of rats. *denotes that increase in $CdCl_2$ is statistically significant ($p<0.05$) when compared to control rats.

In addition, levels of $CdCl_2$ in the testes of animals that were challenged with $CdCl_2$ and water only increased significantly ($p<0.05$) when compared to the control animals that were given water only. Animals that were challenged with $CdCl_2$ and supplemented with green tea or black tea recorded a significant ($p<0.05$) reduction on $CdCl_2$ levels in the testes when compared with animals that were treated with $CdCl_2$ and water only. Black and green teas were more potent and better at reducing the levels of $CdCl_2$ in the testes than EDTA. Likewise, the $CdCl_2$ levels in the animals that were challenged with $CdCl_2$ and supplemented with black or green tea were almost at the same level as those of the control animals that received water only as shown in Figure 31.

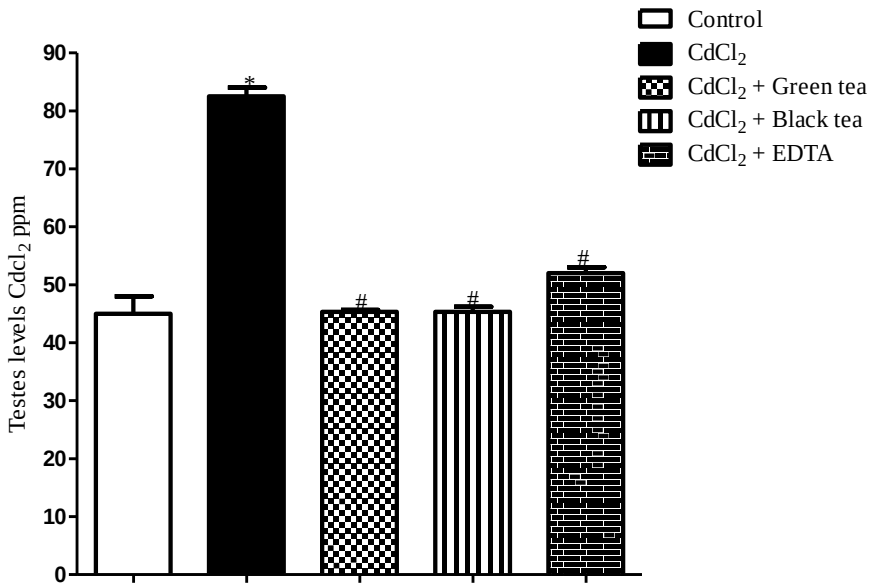

Figure 31: Effects of tea and EDTA on Levels of $CdCl_2$ in the testes of rats.

#denotes that green/black tea and EDTA significantly (p<0.05) reduced $CdCl_2$ levels in the testes of rats. *denotes that increase in $CdCl_2$ is statistically significant (p<0.05) when compared to control rats.

Finally, levels of $CdCl_2$ in the bones of animals that were challenged with $CdCl_2$ and water only increased significantly (p<0.05) when compared to the control animals that were given water only. Animals that were challenged with $CdCl_2$ and supplemented with green tea or black tea recorded a reduction on $CdCl_2$ levels in the bones by 9.52% and 5.7% respectively when compared with animals that were treated with $CdCl_2$ and water only. Green tea was more potent and better at reducing the levels of $CdCl_2$ in the bones than EDTA, the conventional synthetic chelating agent as shown in Figure 32.

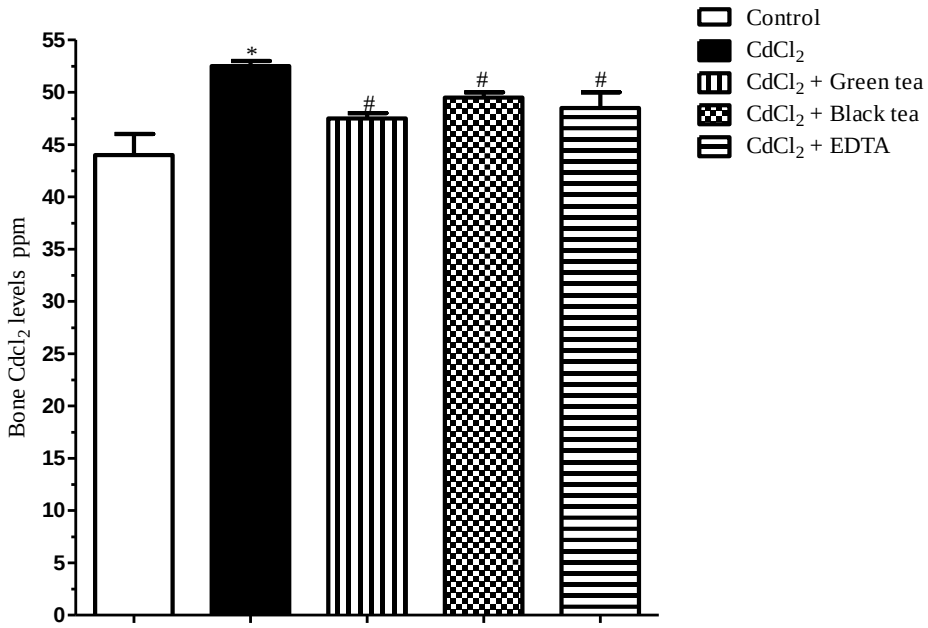

Figure 32: Effects of tea and EDTA on Levels of $CdCl_2$ in the long bone of rats.

#denotes that green/black tea and EDTA significantly (p<0.05) reduced $CdCl_2$ levels in the long bone of rats. *denotes that increase in $CdCl_2$ is statistically significant (p<0.05) when compared to control rats.

4.10 Histopathology and Immunohistochemistry

At the end of the study, tissues were sectioned and stained by the Hematoxylin and eosin method (H&E).The pathological observations of the brain of the control rats were with no disruption of brain tissue, inflammatory cells or activation of microglia. The brain of the rats that were treated with $CdCl_2$ showed pathological changes that were presented as marked activation of microglia, brain tissue disruption, lymphocytic inflammatory infiltration, generalized oligodendrocytes. Both black and green tea ameliorated the effects of $CdCl_2$ with a noticeable reduction in inflammatory cells and a reduction in microglia with normal brain architecture that was comparable to that of the control group that received water only as shown in Figure 33 and Figure 34.

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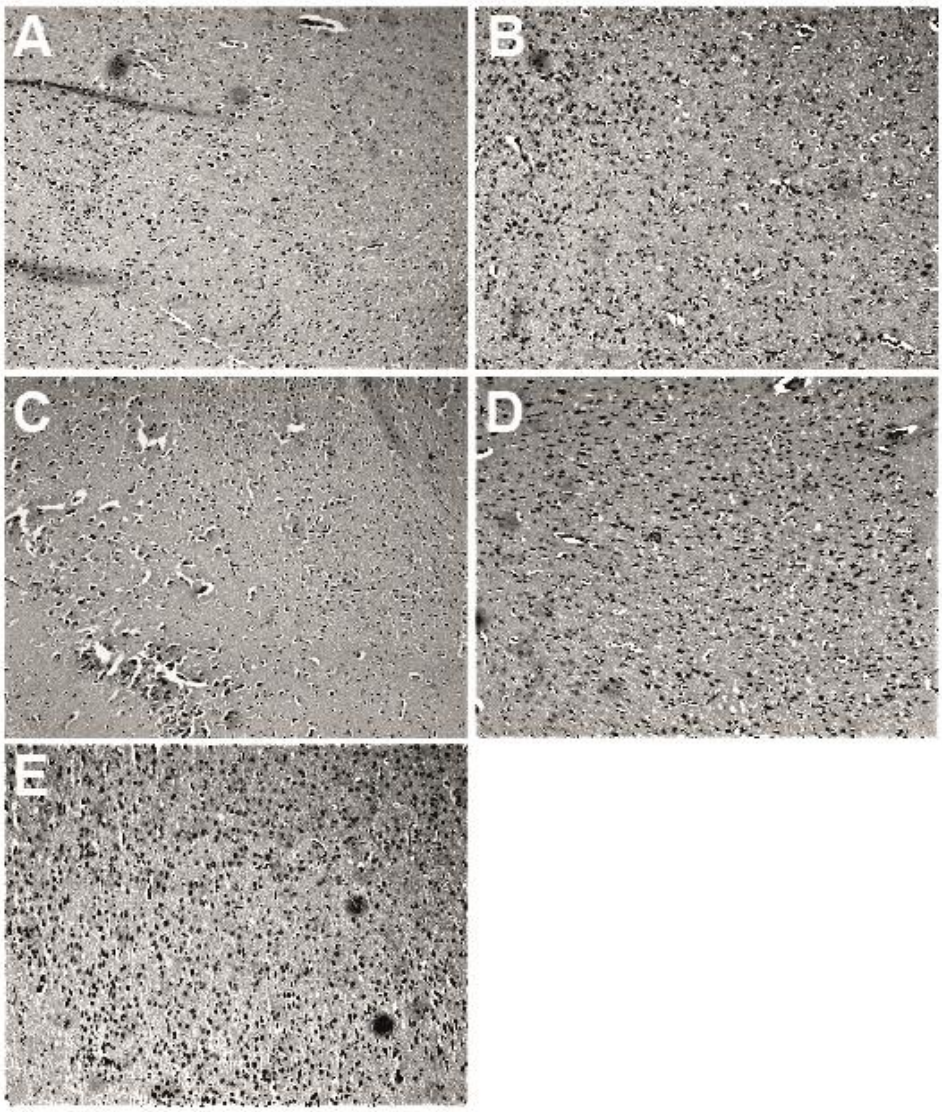

Figure 33: Representative photomicrograph showing histopathological changes in the rat brain.

Plate A: Brain of normal health rat (control), Plate B: Brain of $CdCl_2$ treated rat, Plate C: Brain tissue of rat treated with $CdCl_2$ and Green tea, Plate D: Brain tissue of rat treated with $CdCl_2$ and Black tea, Plate E: Brain tissue of rat treated with $CdCl_2$ and EDTA (x200).

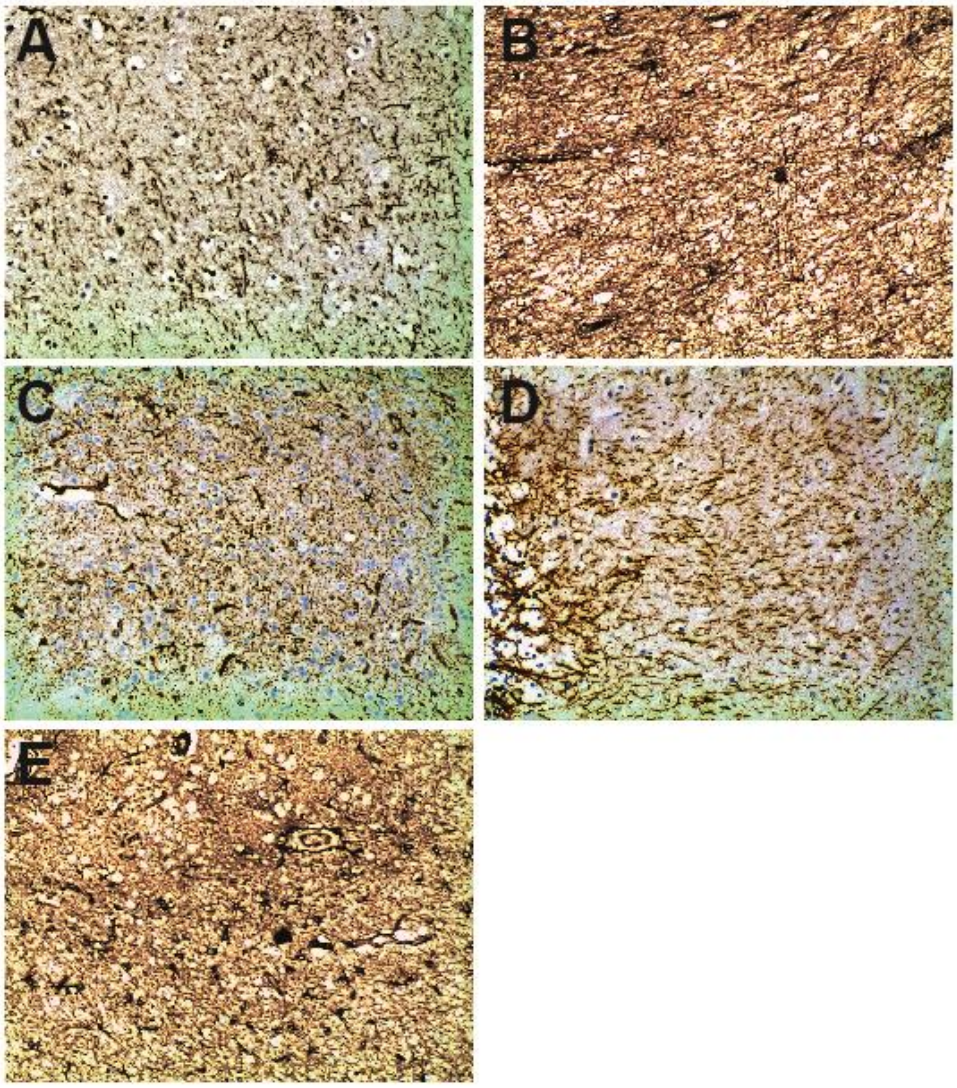

Figure 34: Representative photomicrographs showing the effects of Cd, BTE, GTE and EDTA on the immunohistochemistry of the brain.

Plate A: Normal brain of healthy rat (control), Plate B: $CdCl_2$ challenged brain section, Plate C: Brain section of Cadmiumand Green tea treated rat, Plate D: Brain section of Cadmium and Black tea treated rat, Plate E: Brain section of Cadmium and EDTA treated rat (x 200).

The pathological observations on the testes of the control rats were normal. The histological structure of the seminiferous tubules, spermatogenic cells and interstitial cells were normal in architecture. However, there was marked pathological changes in the rats that were treated with $CdCl_2$ and water only. These included odema, degeneration of spermatogenic cells and congestion of blood vessels. Additionally, $CdCl_2$ induced a noticeable modification of the spermatogenic process with intense reductions on the spermatozoa production in lumen of the seminiferous tubules. Plausibly, both black tea and green tea ameliorated the aforementioned

immense pathological changes. The spermatogenic and interstitial cells showed a great improvement as shown in Figure 35.

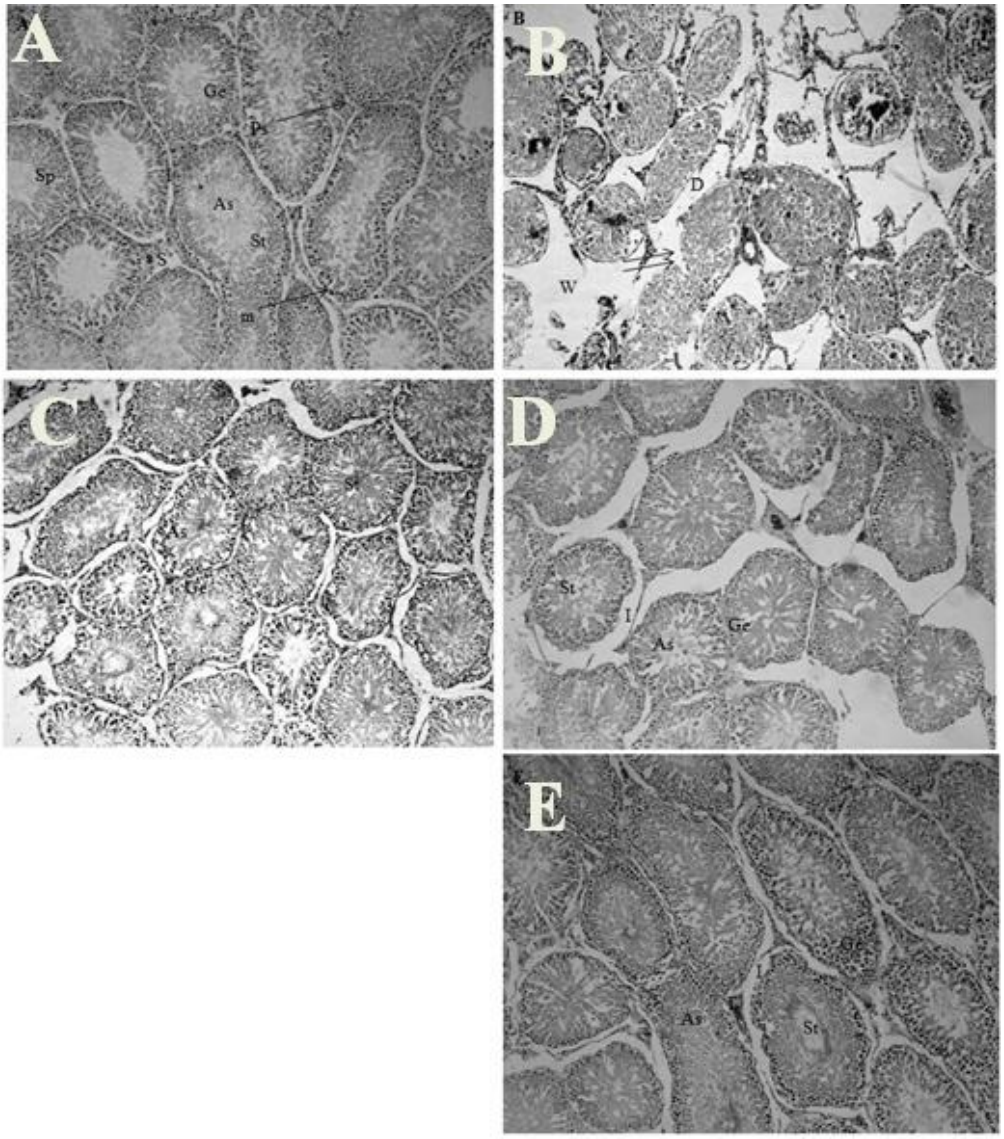

Figure 35: Histological sections of adult albino rats testes sections

Slide A; Normal pathological features of the Seminiferous tubules (St), Aggregations of sperms (As) as seen in their lumina, Germinal epithelium (Ge), Narrow interstitial space (I) showing clusters cells and leydig cells, Spermatogenic cells such primary spermatocytes (Ps), Sertoli cells (s and arrow) resting on regular basement membrane, Spermatogonia (Sp) The tubule is ensheathed by a single layer of myeloid cells (m). **Slide B**; $CdCl_2$ treated group showing multiple shrunken tubules (Double arrows) with other tubules showing different morphologies with superficial reduced layers of germinal epithelium. Some tubules are resting on an irregular basement membrane with sloughing of germ cells (Single arrow) and

aggregation of sperm (As). Additionally, wide lumina (W) and wide interstitial space are visible and damaged D membrane, **Slide C**; $CdCl_2$ group treated with green tea showing most of the seminiferous tubules (St), having nearly regular contour and are lined with stratified germinal epithelium (Ge). Their lumina contain aggregations of sperms (As) and relatively narrow interstitial spaces (I) with clusters of cells. **Slide D**; $CdCl_2$ group treated with black tea showing most of the seminiferous tubules (St), having nearly regular contour and are lined with stratified germinal epithelium (Ge). Their lumina contain aggregations of sperms (As) and narrow interstitial spaces (I) with clusters of leydig cells. **Slide E**; $CdCl_2$ group treated with EDTA showing seminiferous tubules (St) with disorganized epithelial lining and lumina of the tubules are filled with degenerated germ cells and relatively stratified germinal epithelium (Ge) with degenerated germ cells and relatively narrow interstitial space (I) with showing clustered leydig cells (L). (H&E x 200)

Furthermore, the hepatic parenchyma of the placebo group exhibited numerous hepatic lobules detached by very slight connective tissue septa enclosing the triad (Figure 36). Administration of $CdCl_2$ caused severe liver injury as well as fatty variations, hemorrhage, lymphocytic perivascular cuffing, congestion of blood vessels, focal necrosis and proliferation of kupffer cells and bile ductless. These pathological changes were ameliorated in the liver of rats supplemented with either black tea or green tea where the hepatocytes looked normal while the proliferation of bile ductless and dilation of portal vein were visible. Although the liver was generally normal in rats supplemented with EDTA, the improvement of hepatocytes was partial with minor focal lymphocytic infiltration and necrotic area, few lipid droplets and perivascular cuffing as sown in figure 36.

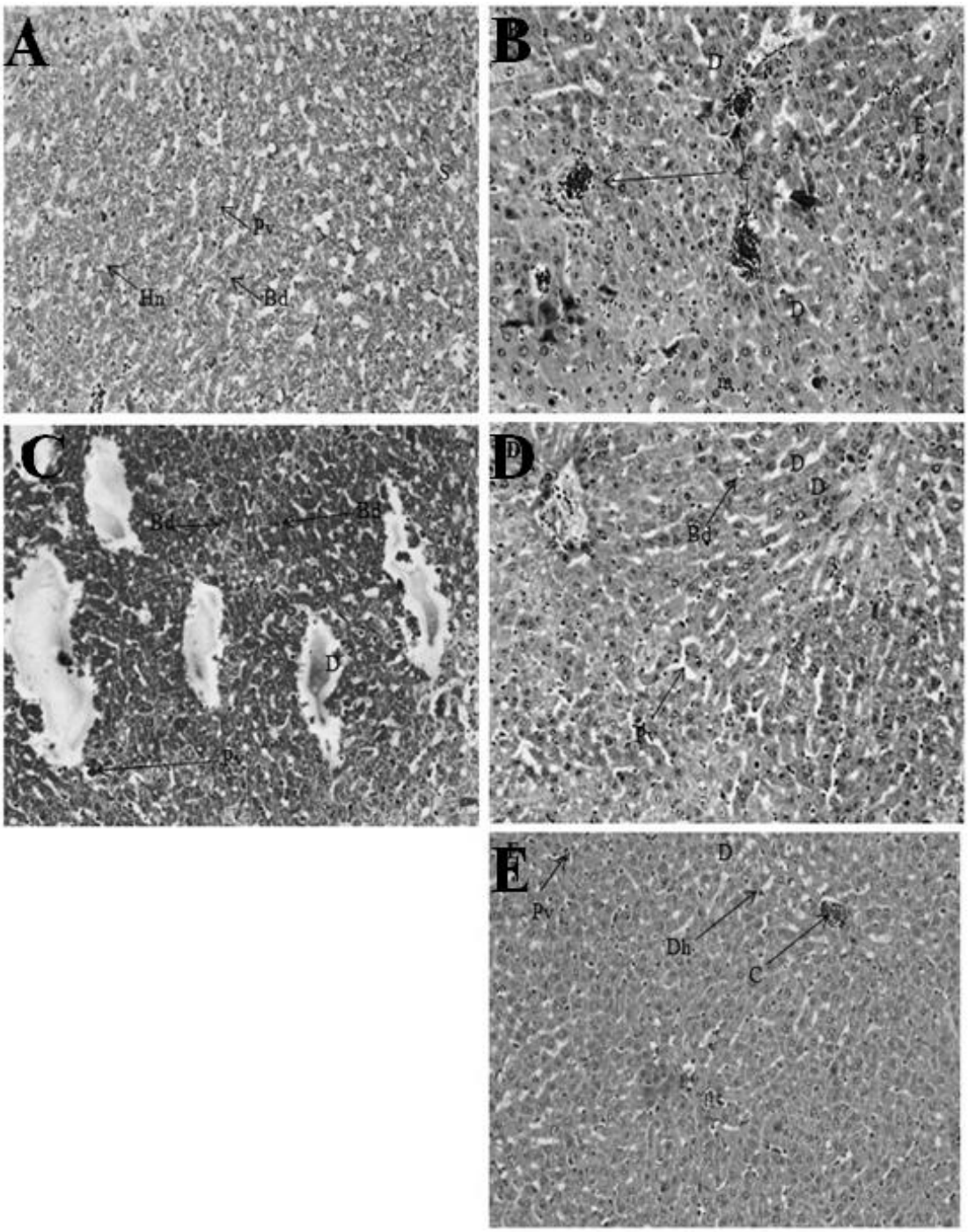

Figure 36: A photomicrograph of Albino rats liver

Slide A; Hepatic tissue of the control rat groups showing normal hepatic architecture, hepatocyte with nuclei (Hn), sinusoids (S), portal vein (Pv) and portal tract with Bile ductless (Bd). **Slide B**; Hepatocyte cytoplasm, foamy and filled with vacuoles is light with blurred trabecular structure of the liver. Cell sizes are enlarged E, nuclear chromatin is more compact and fatty droplets D visible,hepatic congestion C with wide spread necrobiotic changes of the hepatocytes and focal area of coagulative necrosis of hepatocytes surrounded by inflammatory

cells, slightly smaller nucleoli are conspicuous: Necrosis of single hepatocytes- nuclei are contracted pycnotic with condensed chromatin. Accumulation of mononuclear cells (m) in the vicinity of sinusoids (S), the sinusoid walls shows numerous kupffer cells. **Slide C**; Hepatic tissue of $Cdcl_2$ rats treated with green tea showing normal hepatocyte, dilation of the portal vein (Pv), proliferation of bile ductless (Bd) and few fatty D droplets . **Slide D**; Hepatic tissue of $Cdcl_2$ rats treated with black tea showing normal hepatocyte, congested portal vein (Pv), proliferation of bile ductless (Bd) and few fatty (D) droplets. **Slide E**; Hepatic tissue of $CdCl_2$ rats treated with EDTA showing few fatty droplets D, proliferation of bile ductless (Bd), and distinct vacuolar degeneration of numerous hepatocytes, congested branches of the portal vein (Pv) in the portal area, some degenerated hepatocytes are completely destructed (Dh) and obscured. (H&E x200)

Moreover, there were no pathological changes in the kidney of the control rats that received water only in the study. The histological structure of the renal corpuscles presented a tuft of blood capillaries surrounded by the Bowman's capsule while the renal tubules encompassed proximal convoluted tubules lined by large pyramidal cells with a brush border along the distal convoluted tubules with smooth cuboidal cells. Rats treated with $CdCl_2$ indicated major pathological changes of degeneration of the glomerular tuft, cytoplasmic degeneration of the cells of the renal tubule, dilation and congested blood vessels, focal infiltration of macrophages and lymphocytes at the medullary sinus. Remarkably, both black tea and green tea were able to reduce significantly the lesions induced by $CdCl_2$.The glomeruli and renal tubules looked approximately similar to the control rats that received water only. On the other hand, rats that were supplemented with EDTA presented a slight improvement of the glomeruli and tubules as shown in Figure 37.

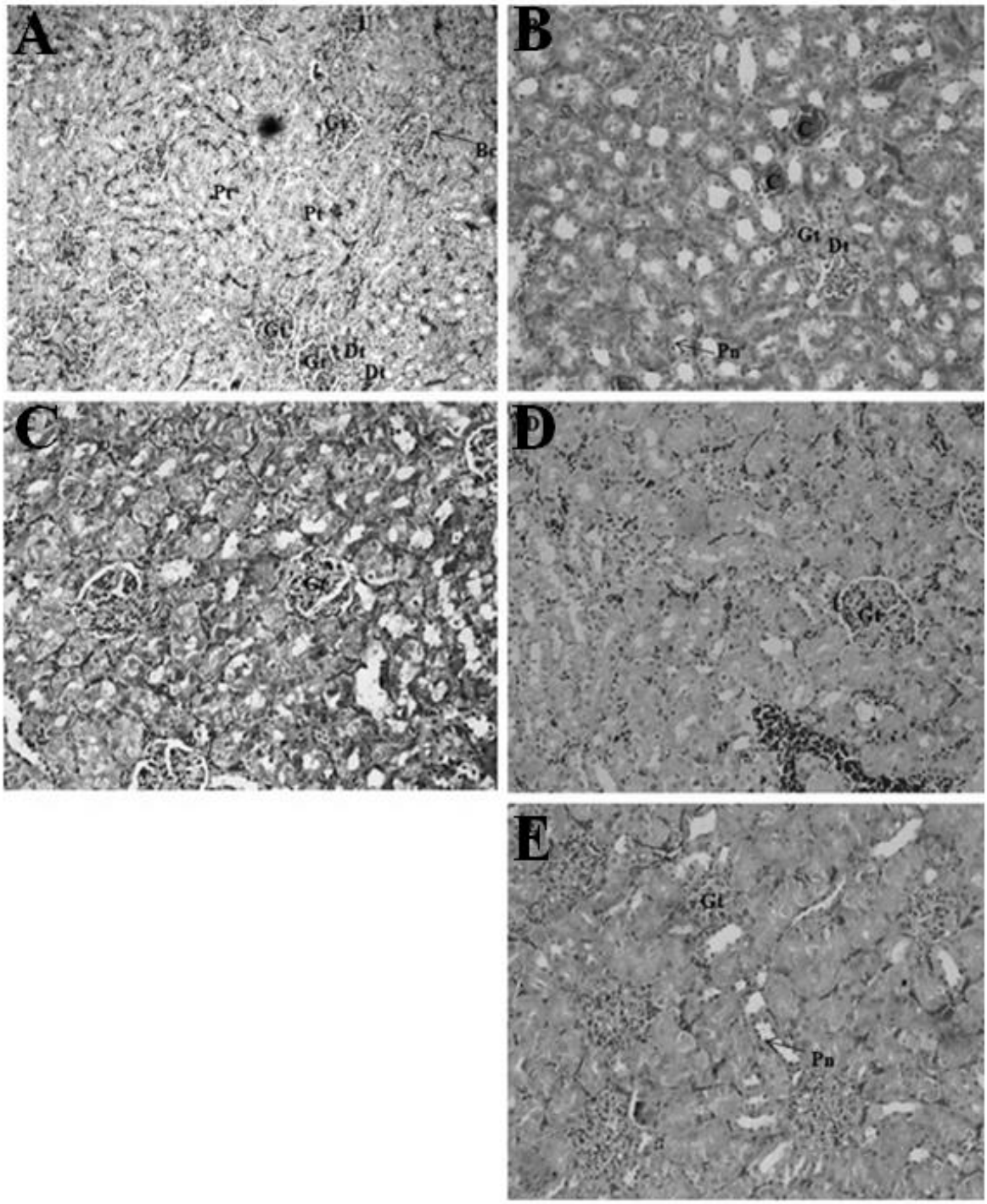

Figure 37: Cross sections of the kidneys cortex region

Slide A: Renal tissue of the control group demonstrating normal appearance of the glomerular tuft (Gt), Urinary space (U), Bowman's capsule (Bc), proximal tubules (Pt), Distal tubules (Dt) and their nucleus (N). **Slide** B; Renal tissue of $CdCl_2$ treated rats showing disruption of the Bowman's capsule (Db) , degenerated cytoplasm of some cells of the renal tubules and some tubules are necrotic showing pyknotic neclei (Pn) and multiple foci of hemorrhage and congestion (C), there is also shrinking and degeneration of glomeruli (Gt) and inflammatory

cell infiltrations. **Slide C;** Renal tissue of rats treated with $CdCl_2$ and supplemented with green tea improvement of the glomerular tuft and renal tubules with minor focal reactions.**Slide D;** Renal tissue of rats treated with $CdCl_2$ and supplemented with black tea improvement of the glomerular tuft (Gt) and renal tubules with minor focal reactions. **Slide E**; Renal tissue of rats treated with $CdCl_2$ and supplemented with EDTA showing improvement of the glomerular tuft and degenerated cytoplasm of some cells of the renal tubules(H&E x 200)

4.11 Discussion

4.11.1 Tea flavonoids of Kenyan tea cultivar

In the current study, the levels of total polyphenols, metals, total theaflavins and antioxidant capacity of both aerated and unaerated teas processed from cultivar TFRK 6/8 were determined. This study compared the total polyphenols levels in the black and green tea used on the animal model study.The green (unaerated) and the black (aerated) differed significantly in the levels of total polyphenols ($p \leq 0.05$). Total polyphenol content of aerated and anaerated teas processed was 20.4% and 25.0% respectively. Total polyphenols which are constituents of secondary metabolism in plants remained almost intact in unaerated processed teas, since the enzyme polyphenol oxidase is inactivated by heat during the early stages of processing (Ingrid *et al*., 2012). It can therefore be deduced that green tea polyphenols consist of simple and complex compounds, the large majority of which are the flavonoid monomers catechins, catechin gallates and flavonols (Jenny *et al*., 2012; Shitandi *et al*., 2012).

On the other hand, black tea usually consists of residual green tea polyphenols such as catechins, flavonols and oxidation products of green tea polyphenols such as theaflavins and thearubigins (Shitandi *et al*., 2012). Studies have shown that various catechins and catechin gallates may be epimerized or degallated during the processing of black tea where most of the catechins and their gallates undergo known enzymatic oxidation to form theaflavins and thearubigins (Obanda *et al*., 2001; Shitandi *et al*., 2012). Therefore, the amount of polyphenols in green tea is higher than that of black teas since the auto-oxidation results to significant conversion of the polyphenols to highly polymerized molecules; thearubigins and theaflavins (Karori *et al.,* 2014).

Results from this study also revealed that the total catechins levels were statistically different ($p<0.05$). Black (aerated) teas had lower catechin levels than the green (non-aerated) teas. Individual catechins varied significantly ($p<0.05$) among the teas with EGCG, GC and EGC levels being the highest and +C, ECG and EC being less abundant. These results are in agreement with those of Karori *et al*., (2007). This clearly shows that the auto-oxidation step

of black tea processing also interfered with the total catechins content of the final product. Other studies have found out that during the manufacture of black tea, the polyphenol oxidase enzyme catalyzes the oxidation of catechins into quinones by molecular oxygen (Obanda *et al.,* 2001; Selena *et al.,* 2012). In the aforementioned process, the quinones further condense to form theaflavins, thearubigins, bisflavonols and other complex oligomers (Riemersma *et al.,* 2001; Yashin *et al*., 2015). On the contrary, green tea is made by inactivating the fermentation process by passing the freshly harvested tea leaves in steam or pan firing treatment (Sabu *et al.,* 2002), leaving catechins almost intact. These processing methods of tea give forth to preparations with different chemical configurations and different pharmacological properties.

The HPLC catechins profiling revealed that the elution time and the order of elution of individual catechins was as follows; Gallic acid (GA), Epigallocatechin (EGC), Catechin (C), Caffein (CAFF), Epicatechin (EC), Epigallocatechin gallate (EGCG) and Epicatechin gallate (ECG). The highest peaks were recorded for EGCG EGC and ECG respectively, a result that corroborated with earlier studies (Karori *et al.,* 2014). However, alongside the key peaks identified, several minor peaks were also fractionated; indicating that other unidentified catechins existed in the tea extracts (Figure 1 and 2). There was however great similarity in the HPLC chromatographic pattern which indicated the close similarity in catechin profiles in both black and green tea studied. A similar observation was made by Karori *et al*., (2014)

The levels of theaflavins and thearubigins which are the main products of fermentation increased in black tea when compared to green tea. Black tea recorded 2.19% and 16.13% of TFs and TRs, respectively while the levels of TFs and TRs in green tea were 1.46% and 12.36%, respectively. This is in line with other studies which cited the release of polyphenol oxidase that interacts with phenolic compounds to form theaflavins and thearubigins (Reeves *et al.,* 1987; Mahanta *et al.,* 1992; Karori *et al.,* 2014).

The two types of processed tea leaf used in this study contained some amount of metals (Table 3). Aluminum was the most predominant and magnesium was the lowest. The study showed that tea is a good source of manganese, copper and calcium in diet as their levels match the acceptable daily intakes and do not therefore expose the tea user to high levels which can be toxic. The nonessential elements including aluminum, lead and cadmium were in very low concentrations in tea infusions and thus do not pose any risks in terms of toxic metals in diet, with their contents matching permissible levels for toxic metals in food and beverages. These results agree with other studies which suggested that tea grown and marketed in Kenya is potentially rich in dietary source of some essential minerals including Fe, Zn and Cu and had

Fe, Zn, Cu, Pb and Cd levels well within the international (Maximum Permissible Concentrations) MPC's set for tea (Moseti *et al*., 2013).

One of the areas of research on tea that has elicited a lot interest is its antioxidant capacity (Mandel *et al*., 2004; Karori *et al*., 2007; Ingrid *et al*., 2012; Kopjar *et al*., 2015). This is due to the beverages potential of being a free radical scavenger that ends up boosting the antioxidant status of the biological system (Kodama *et al*., 2010). In line with this fact, the antioxidant capability of both green and black teas that were used in this study was determined. Overall, green tea recorded higher percent antioxidant capacity than black tea, that is, 88.7% and 77.6%, respectively. The high antioxidant capacity of green tea could be related to its high levels of free catechins. This is in line with other studies that have correlated between high antioxidant activity in tea extracts with high levels of EGCG, EGC and ECG (Gramza *et al.*, 2006; Karori *et al*., 2014). The availability of phenolic hydroxyl groups in the structures of catechins confers tea with the ability of being a potent free radical scavenger (Amie *et al.*, 2003). It has been hypothesized that the most potent antioxidants are catechins that possess a 3'4' and 5' –trihydoxlated substitution pattern on the B ring and/or hydroxyl group at the C-3 position of the catechins structure (Karori *et al.,* 2014). This stabilizes the catechins phenoxyl radical through participation in electron delocalization which is a very important phenomenon in anti-radical potential.

Although tea has for a long time been well-thought out as a beverage, current information shows that many people are starting to think of it as a potent medicinal compound that is endowed with numerous health benefits (Xiang *et al.*, 2016). This is due to its biological and pharmacological activities that have potential health benefits to humans (Mandel *et al.*, 2006; Tavares et *al*., 2011; Xiang *et al*., 2016). These prospective health benefits of tea have been attributed to their elevated levels of catechins, thearubigins and theaflavins (Rietveld *et al.*, 2003). However, there is still a paucity of data on its ability to chelate heavy metals and reduce their burden in the biological system. This study sought to establish the ability of tea to ameliorate the toxic effects of $CdCl_2$ administered to a rat model. Tea used in this study was generally found to reduce the effects and levels of $CdCl_2$in the tissues that were studied. This was inferred to be due its ability to interrupt auto-oxidation and confer protection against deleterious metal mediated oxidative stress through chelating metal ions, inhibiting formation of free radicals, breaking the auto-oxidative chain reactions and reducing localized oxygen concentrations (Brewer, 2011). Perhaps, the chemical potency of tea and its lipophilic nature made it access peroxy radicals especially in membrane, micellar and emulsion systems as has been postulated by Wanatabe *et al.,* (2010).

4.11.2 Effects of tea on the liver function and ZHX1 rats

All the biochemical markers including total proteins, albumin, ALT, AST, ALP, MDA and ZHX1 investigated in the rats that were administered with either black tea or green tea aqueous extracts in this study were unaltered when compared with the controls that received water only. This is a clear indication that the teas used in this study were none toxic and were well tolerated by the rats. Other studies have documented that tea is rapidly absorbed and distributed into the mucous membrane of the small intestines, the liver and most importantly it crosses the blood brain barrier (Smith *et al.,* 2011; Khalid *et al.,* 2014). This phenomenon makes tea more superior to the synthetic chelating agents such as EDTA which has been shown to be poorly absorbed and distributed in the extracellular fluids, limiting its ability to mobilize and remove these metals from the inside of the cells (Klaassen, 2006). Furthermore, EDTA has been shown to redistribute metals from other tissues to the brain (Flora *et al.,* 2010).

In this study, tea caused minimum body weight gain with few fluctuations throughout the experimental period in the rats used. This body weight gain was relative to the initial body weight of the rats. Similar observations were made by Kao *et al.,* (2000) who inferred that, body weight gain or loss is dose dependent. However, the exact role of the catechin metabolites and catabolites in enhancing fat oxidation is unknown. Future *in vitro* investigations should use only physiologically relevant doses and catechin compounds (conjugated) to specifically identify realistic *in vivo* effects of tea extracts(Adrian *et al*., 2013).

Furthermore, tea also enhanced a drop in percent PCV levels following cadmium chloride administration in rats. Indeed, studies have shown that tea interacts with iron forming insoluble complexes and consequently reduces levels of Hb (Hunt and Roughead, 2000). More studies have established that increased consumption of black tea significantly inhibits hemoglobin biosynthesis through its effects on iron absorption (Imai and Nakachi, 1995; Thankachan *et al.,* 2008). Rashid et al., (2014) attributed the reduction of PCV levels to a reduction in erythrocytes synthesis following anthocyanin administration. It is therefore, hypothesized that the enhanced reduction in percent PCV levels in the current study would be due to the presence of the aqueous tea extracts that directly affected the already affected iron metabolisms by cadmium. This affected red blood cell synthesis, hence a reduction in the percent PCV.

The current findings indicate that both extracts of tea has no deleterious effects in rats and have powerful antifibrotic potential than EDTA. These observations corroborate with those of other studies that have shown the dimerits of EDTA such as its inability to penetrate the membranes, its effects of redistributing other metals to the brain and the limitation of its action

to extracellular membranes (Flora *et al*., 2010); its chelation of zinc that confers effects on the structure and interactions of zinc binding proteins that leads to irreversible denaturation and aggregation of these proteins (Nyborg *et al*., 2004). EDTA also causes renal toxicity and can deplete essential minerals (Aposhian *et al.,* 1995).

The tolerability of tea by rats could be attributed to its wide range of biomolecules such as enzymes, proteins, carbohydrates such as cellulose, pectins, glucose, fructose, sucrose, amino acids such as theanine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine arginine and leucine (Chako *et al*., 2010); minerals and trace elements (Moseti *et al*., 2013). Its ability of neutralizing free radicals (Dulloo *et al.*, 1999), and E recycling ability (Intra *et al.*, 2007), may partly account for the many protective effects of tea in the biological system.

4.11.3 Cadmium chloride mediated effects on different tissues of rats

In this study, treatment of rats with cadmium chloride caused a significant increase of the activities of serum AST, ALT, ALP and an increase of GSH and TBARS in both the brain and liver tissue homogenates. Furthermore, there was a decrease in brain and liver ZHX1. Subsequently, cadmium chloride caused a decrease in serum total proteins, albumin and body weight. There were also significant and severe histological changes such as severe hepatocyte necrosis, fatty changes, signs of cell degeneration and inflammatory cell infiltrations in the liver, shrinking and degeneration of the glomeruli including pyknosis and vacuolated cytoplasm in the kidney, shrinkage of the neurons and excessive proliferation of inflammatory cells, extensive hemorrhage in the cerebral cortex, numerous pyramidal cells with pyknotic nuclei, microglial cells with cytolysis in the brain and degeneration of spermatogenic cells, oedema, hemorrhage, congestion and multifocal areas of ischemic necrosis in the testes. The alterations of the aforementioned biochemical and histopathological parameters indicate oxidative injury to the liver, brain, kidney and the testes of the cadmium treated rats. Furthermore, cadmium tissue burden was also evident by the elevated levels of cadmium in the liver, bone, testes and the kidney of the rats. These findings corroborate with studies by Renugadevi and Prabu (2010), who found significantly increased levels of AST, ALT and ALP during cadmium induced toxicity; indicating degenerative changes and hepatic cell damage (Jaramillo-Jurez *et al.*, 2008; Adebajo *et al.*, 2009). Significant increase of ALT levels is clear indications of hepatotoxicity (Renugadevi and Prabu, 2010) while the increase in ALP which is located with both sinusoidal and bile canacular membranes represent general hepatic toxicity (Naik, 2010). Moreover, research has shown that continuous exposure of the liver to cadmium

leads to hepatotoxicity that reduces the endogenous antioxidant system and alters metabolism (Oyinloye *et al.,* 2016). This is due to the ability of cadmium to form a complex with the cysteine rich metallothionein protein (Cd-MT) in the liver (Klaassen *et al*., 1999). This complex impairs biomarkers of the liver function (Adikwu *et al.,* 2013), after which it gets slowly released from the liver and circulates in the kidney (Prabu *et al.,* 2010). There is a close link between appearance of the functional enzymes in the blood stream and lipid peroxidation which was exhibited in the current study by increases in TBARS. This collaborates with studies that have already shown that Cd causes tissue peroxidation with subsequent liver dysfunction (Lakshmi *et al.,* 2012). The accumulation of hydroperoxides has been shown to cause cytotoxicity which in turn causes peroxidation of membrane phospholipids, the basis of hepatocellular damage and subsequent appearance of the functional enzymes in the blood stream (Renugadevi and Prabu, 2010).

This study further observed a significant reduction in total protein and albumin levels in the serum of rats. The reduction in total protein content in the serum of the experimental rats could be attributed to diversification of energy for detoxification and/or reduced synthesis due to liver tissue damage (Moshage *et al*., 1987; Nicholson *et al*., 2000; Kaysen *et al*., 2001). This is in line with previous studies that observed a significant reduction of total proteins and albumin levels in rats exposed to cadmium (Bamidele *et al.,* 2012). These effects were due to oxidative damage to proteins (El-Demerdash *et al.,* 2004) by binding to their reactive SH-groups (Vallee and Ulmer, 1972). Studies carried out in fish exposed to cadmium found out that this heavy metal could change protein reserves in fish by affecting liver enzymes that have roles in protein metabolism thus altering the pathways of protein metabolism in tissues and organs (Siddiqui *et al.,* 2014). Albumin represents 55-65% of total protein and is synthesized in the liver at a rate that is dependent on protein intake; its regulation is through feedback by the plasma albumin level (Fahid Al-Hashem, 2009). In the current study there was a significant decrease in serum albumin levels of rats treated with cadmium chloride alone as compared to the control rats that received water only indicating poor liver functions or impaired synthesis, primarily due to liver cells damage or due to diminished protein intake and reduced absorption of amino acids caused by a malabsorption syndromes or malnutrition. On the other hand, it may be that cadmium caused nephritic syndrome and chronic glomerulonephritis as reported earlier in cadmium exposed rabbits (Hristev *et al.,* 2007; Hassan *et al.,* 2012). These authors attributed the decrease in albumin to cadmium adverse effects on the liver such as focal hemorrhages in the liver parenchyma which eventually resulted in decreased protein and albumin synthesis from the hepatocytes. Another possible reason for the decrease of albumin

may be due to cadmium induced impairment of the reabsorptive and secretory functions of renal proximal tubule which results into albuminuria and subsequent hypoalbuminemia (Gena *et al.,* 2010).

The body weight of the cadmium treated rats decreased continuously up to the last day of the experimental period. This observation is in agreement with other studies that have demonstrated that oral administration of cadmium causes a decrease in the body weight of rats (Gathwan *et al.,* 2012; Lakshmi *et al.,* 2012; Prabu *et al.,* 2012). The decreased body weight can be attributed to the heavy metals impairment of glucocorticoid system which plays a very vital role in glucose regulation as well as lipid, carbohydrate and protein metabolisms (Kaltreider *et al.,* 2001).

Cadmium chloride caused anemia that was evident with the reduction in the percent PCV levels of the rats that were used in this experiment. This observation is in agreement with other studies that attributed such a decrease to oxidative stress that results into severe anemia or lethal and malignancies of hematopoietic tissues due to increased fragility and progressive destruction of red blood cells (Balani *et al.,* 2011). Anemia due to cadmium toxicity may also be linked to the effect of cadmium to aminolevulinic acid dehydratase (ALAD) a key enzyme in heme biosynthesis as has been shown in polychlorinated biphenyls (PCBs) toxicity (Selvakumar *et al.,* 2013). Cadmium toxicity may also inhibit the conversion of coproporphyrinogen III to protoporphyrin IX leading to reduction in hemoglobin production and shortened life span of erythrocytes as observed in lead toxicity (Selvakumar *et al.,* 2013)

On the contrary, to what has been reported on the response of GSH to cadmium toxicity, this study observed a significant elevation of GSH in the liver and brain tissues of rats exposed to Cd. This indicates that this non protein thiol plays a very crucial function in the cell's defense against oxidative stress due to Cd toxicity, serving as an additional cytoprotective mechanism through direct metal binding, detoxification of reactive oxygen and the maintenance of cellular redox status (Meister and Anderson, 1983; Ringwood and Conners, 2000). It can be postulated therefore that, this response was propagated by GSH being a potent reductant and cofactor for glutathione peroxidase (GP_X) and glutathione-S-transferase (GST) catalytic reactions (Forman *et al.,* 2009). This subsequently, became one of the cell's coping mechanisms against oxidative stress (Franco *et al.,* 1999). The oxidative stress prompted increase in GSH synthesis to boost endogenous antioxidant system affected by cadmium toxicity (Meister and Anderson, 1983; Ringwood and corners, 2000).

Moreover, cadmium caused a significant decrease in ZHX1, in the liver and the brain of the rats. This would be attributed to an interaction of cadmium with non-coordinating

cysteines in zinc finger proteins with subsequent alterations in the functioning of the protein (Hanas *et al.,* 1996). Consequently, cadmium might have displaced zinc from its coordinating position at the zinc finger (Znf) motif and disturbed DNA binding as well as in the DNA repair proteins (Hartwig, 2001). This effect of cadmium on ZHX1 would modulate gene expression and interfere with signal transduction pathways that are involved in cell growth and development, a typical distinctive feature of cancer (Valko *et al.,* 2006). Furthermore, it would interfere with deregulation of cell proliferation by activating numerous transcription factors, controlling cell cycle and apoptosis (Evan and Vousden, 2001).

In addition, cadmium levels increased significantly in the liver, kidney, testes and bone after 42 days of cadmium exposure to the rats when compared to the control group. This is in agreement with studies that observed a significant increase in cadmium concentration in the liver, kidney, musculature and gills of fish exposed to cadmium for 30 days (Osman *et al*., 2013). Haouem *et al* (2007) made the same observations of cadmium bioaccumulation in tissues. This could be attributed to the predominat binding of Cd to metallothionein forming cadmium metallothionein complex (Cd-MT) (Hamer, 1986). This complex is then distributed to various tissues and organs and finally reabsorbed in the kidney tubules (Ohta and Cherian, 1991). Subsequently,cadmium accumulates in the tissues due to its longer biological half-life (Valko *et al.,* 2011).

Histologically, cadmium caused changes in the liver such as severe hepatocyte necrosis, fatty changes, degeneration signs and inflammatory cell infiltrations. The hepatocytes were enlarged, irregularly arranged and with disorganization of the hepatic architecture. They further increased in size and looked light and foamy with the cytoplasm being filled with numerous vacuole-like spaces. The central vein appeared dilated and congested with immense hemorrhage. This presentation indicates that Cd caused severe oxidative stress that was injurious and damaged the liver. This is in agreement with previous studies that reported toxicity of Cd to the liver (Borges et al., 2008; Ersan *et al.,* 2008; Renugadevi and Prabu, 2010). These authors elucidated that subchronic administration of Cd caused liver damage and demonstrated histopathological alterations in addition to moderate degeneration and discrete necrosis. They further elucidated that Cd causes vacuolar degeneration and increased density of the nuclear chromatin with very compact nuclear structure found in hepatocytes. The necrosis was more visible in the centrilobular and extending through the whole liver, an observation that was also made by Sinha *et al.,* (2009). The mechanisms of Cd induced histohepatotoxicity have been explained by a number of authors, who have suggested that Cd disturbs membranes integrity, elicits cytotoxic and inflammatory mediators, generates reactive

oxygen species which cause oxidative damage to lipid contents of the membranes, causes primary injury cells by binding to Cd sulfhydryl groups in the mitochondria and secondary injury initiated by activation of kupffer cells in the liver (Muller *et al.,* 1986; Waisberg *et al.,* 2003;Koyu *et al.,* 2006; Ahmad *et al.,* 2012). The overproduction of ROS would be due to perturbations of the endogenous antioxidants due to the direct action of Cd on peroxidation reaction or iron mediated peroxidation (Waisberg *et al.,* 2003). Furthermore, the toxicological effects of Cd could be attributed to formation of toxic metabolites in case it is activated by cytochrome P_{450} and interference of essential metals (Savides, 1983).

The kidney has been shown to be more predisposed to cadmium toxicity (Jin *et al.,* 2002); causing proximal tubular damage and proximal tubular epithelial cell necrosis (Morales *et al.,* 2006; Garcon *et al.,* 2007; Pari *et al.,* 2007). This was observed in this study where exposure to cadmium caused histological glomerulus and tubular changes in the kidney of the experimental rats. Shrinking and degeneration of the glomeruli including pyknosis and vacuolated cytoplasm were observed. These observations concurred with those of other studies that documented that cadmium toxicity affects the glomerular capillaries sparing Bowman's space atrophy of some glomerulus (Jemai *et al.,* 2010). Other histopathological studies have shown cadmium toxicity causes oedema in the kidney (Choi and Rhee, 2003) as well as proximal tubular necrosis, apoptosis and tubular degeneration (Damek-Poprawa and Sawicka-Kapusta, 2004). The nephrotoxicity caused by cadmium toxicity is thought to occur through cadmium metallothionein complex which is synthesized in the liver, released into circulation and taken up by renal proximal tubule cells (Dudley *et al.,* 1985). The complex in circulation is filtered through the glomeruli in the kidney and taken up by the proximal tubular cells (Sudo *et al.,* 1996). While in circulation in the kidney, the complex causes injury particularly in the cortical region, passing the proximal region and eventually causing a continuing loss of the kidney's integrity (Dorian and Klaassen, 1995; Thijssen *et al.,* 2007). These changes are attributed to the free radicals produced by cadmium that accumulate and cause lipid peroxidation (Renugadevi and Prabu, 2009)

There was also marked histological changes in the testes tissue of the rats that were used in this study. The observed changes were in the form of degeneration of spermatogenic cells, oedema, hemorrhage, congestion and multifocal areas of ischemic necrosis. Similar studies observed that cadmium chloride at low doses (1mg/kg body weight) for thirty days caused lack of spermatogenesis and severe necrosis of the testes of rats (Blanco *et al.,* 2007). Additionally more reports have demonstrated that parenteral doses of cadmium chloride of 2-4 mg/kg body weight had the ability of causing endothelial damage of the small vessels,

oedema and hemorrhage (Santos *et al*., 2004). Several other studies have reported that changes in testicular histopathology can be attributed to testicular blood vessel damage followed by the degeneration of spermatopoietic epithelial as the main cause of cadmium toxicity (Thompson and Bannigan, 2008; Messaoudi *et al.*, 2010). Moreover, cadmium exerts direct effects on testicular vascular endothelium by indirect generation of highly reactive free radicals in the testicular tissues that would cause ischemia, hypoxia and lipid peroxidation. (Aoki and Hoffer, 1978; Zitkevicius*et al.*, 2011).Increased Cd accumulation in the hypothalamus, pituitary and testis decreases plasma levels of follicle stimulating hormone in rats, suggesting a possible effect of Cd on the hypothalamic-pituitary-testicular axis (Lafuente *et al*., 2000). Previous data has also linked reactive oxygen species (ROS) with Cd-induced testicular damage (Farombi *et al*., 2012). Likewise, acute, as well as chronic, Cd exposure is associated with elevated lipid peroxidation in the lung, brain, kidney, liver, erythrocytes, and testis (Bagchi *et al.*, 1997).

It is widely recognized that the brain is highly rich in polyunsaturated fatty acids and therefore it is prone to oxidative stress (Smith *et al.*, 1997). In this study, $CdCl_2$ induced morphopathological changes that involved the cerebral cortex regions in the brain of the rats. The neurons underwent either degenerative changes that were manifested by shrinkage of the neurons and excessive proliferation of inflammatory cells in the cerebral and cerebellar cortices. Additionally, there was observation of extensive hemorrhage in the cerebral cortex, numerous pyramidal cells with pyknotic nuclei, and microglial cells with cytolysis in the brain of rats. These observations are in agreement with other studies that showed extensive hemorrhage in the cerebral and cerebellar cortices, numerous pyramidal cells with pyknotic nuclei, neuroglial cells with cytolysis and altered purkinje cells in the central nervous system (CNS) of rats and rabbits exposed to high doses of cadmium (Wong *et al.*, 1980; Mendez-Armenta *et al.*, 2001). The pathological changes observed in the brain of rats exposed to cadmium may be attributed to the high susceptibility of rats to cadmium toxicity induced oxidative stress (Williams, 1995). Due to the observations made, the study endeavored further to understand the response of the glial cells to cadmium induced toxicity with a keen interest on GFAP expressing astrocytes. This is because astrocytes are a source of neutrotrophic factors and play a very cardinal role in long term potentiation (LTP) (Rudge *et al.*, 1992; Muller *et al.*, 1995; McCall *et al.*, 1996). They are also involved in the maintenance of the BBB integrity (Pekny *et al.*, 1998; Choi and Kim, 2008); and therefore subsequent loss of BBB integrity is connected with astrocyte damage under neuropathological conditions (Prior *et al.*, 2004; Willis *et al.*, 2004). Furthermore, GFAP is an important protein component of the BBB (McCall *et al.*, 1996), that has been recognized as a marker of astrocyte maturation and reactivity

(Calixto*et al.,* 2017). This study observed a marked increase in GFAP following cadmium induced administration to the rats used suggesting cadmium induced neurotoxicity. The astrocytes showed major morphological modifications expressed by their activation. This study further observed that exposure to cadmium reduced astrocyte count, size and number of projections or processes. This corresponds with studies that observed that heavy metals increased the expression of GFAP (Kern *et al.,* 2006), with subsequent increase in apoptosis and morphological alterations in GFAP-expressing astrocytes. Change in number of processes may modulate synaptic activity (McCall *et al.,* 1996). Thus increased GFAP due to cadmium toxicity is thought to interfere with the essential signaling between astrocytes and neurons or synapses. Since elevated GFAP causes astrocyte dysfunction (Quinlan *et al.,* 2007), any therapeutic agents geared towards its decrease during the natural cause of disease is expected to be valuable.

4.11.4 Protective effects of aqueous extracts of black and green tea on Cd induced toxicity in the liver and kidney of rats

The present study revealed that administration of tea extracts significantly ameliorated most signs of cadmium induced toxicity. The perturbations of GSH, increase in lipid peroxidation, impairment of liver functions including biosynthetic capacity, impairment of the transcription repression capacity induced by cadmium chloride were all alleviated in male wistar rats. The antioxidant effect and the marked decrease in the biomarkers of xenobiotics injury shown in the present study by tea extracts is a clear indication that tea may reduce the incidence and severity of toxicological effects of cadmium in rats. In the present study was that tea extracts significantly reduced the levels of cadmium in the liver, kidney, testes and bone tissues and modulated the severe damages that was caused by cadmium. Previous studies seems to suggest that tea could be a stronger antioxidant (Rice-Evans *et al.,* 1995; Abdalla *et al.,* 2009; El-Tohamy *et al.,* 2010); making it a better therapeutic agent in the management of heavy metal toxicity and related conditions.

The protective effect of tea observed here would be mainly due to its high polyphenolic flavonoid levels which have been shown to exhibit an array of biological actions such as radical scavenging, metal chelation and enzyme modulation abilities (Pietta *et al.,* 1998 a, b). It has been widely documented that the polyphenolic composition of tea that may be responsible for its health properties include catechins, flavonols, theaflavins and thearubigins (Yokozawa *et al.,* 2002; Sakata *et al.,* 2004; Chen *et al*., 2005; Cabrera *et al.,* 2006; Wolfram, 2007;Karori *et al.,* 2014). As shown earlier in this study, these biomolecules were found at high levels in the

processed teas of clone TRFK 6/8. Indeed, there is immense scientific evidence supporting the antioxidant, anti-inflammatory, antihypertensive, antimicrobial, antidiabetic and anti-mutagenic properties of tea (Karori *et al.,* 2007;Kerio *et al*., 2012; Santilli *et al.,* 2013; Karori *et al,* 2014; Koech *et al.,* 2014). Inline with the aforementioned pool of health properties of tea, this study provides additional evidence that tea extracts may have stronger antioxidant and metal chelating properties than EDTA. This would be attributed to the enhancement of antioxidant defense system by the tea extracts through modulation of nuclear hepatic transcription factors, inhibition nuclear factor B (NFkB) expression and activation of Nrf2 expression that may be activated and suppressed in the heat stress environment (Sahin *et al.,* 2010), or by scavenging free radicals that are key in lipid peroxidation (Rietveld *et al.,* 2003; Karori *et al.,* 2007; Karori *et al.,* 2014). Additionally, the ability of tea to regenerate alpha-tocopherol by repairing tocopheryl radicals and protection of the hydrophilic antioxidant ascorbate (Mahmoud *et al.,* 2012) is a very powerful mechanism that is also thought to play an immense role in boosting the endogenous antioxidant system. Most importantly, previous studies have reported that tea chelates iron and copper which in turn inhibit further generation of hydroxyl radicals and degradation of lipid hydroperoxides (Azram *et al.,* 2004).

Damaging liver tissue after Cd exposure is a well-known phenomenon, and the obvious sign of hepatic injury is the leakage of hepatic enzymes into plasma. Both the histological appearance and biochemical parameters assayed in this study supported a diagnosis of liver damage by cadmium. The increased levels of serum enzymes such as ALT, AST and ALP and decreased levels of total protein and albumin that ware observed in Cd treated animals indicate the increased permeability, damage or necrosis of hepatocytes (Renugadevi and Prabu *et al*., 2009). Furthermore, the tea extracts used gave a high hepatoprotective effect by reversing the changes caused by $CdCl_2$. The observed decrease in the serum activities of these enzymes showed that tea preserved the structural integrity of the liver from the toxic effects of Cd. It can be postulated that tea achieved this effectiveness by scavenging reactive oxygen species or indirectly through its effects on transcription factors and enzyme activities (Miyagawa *et al.,* 1997; Lung *et al*., 2002). Furthermore, tea inhibits iron-induced oxidation of synaptosomes by scavenging hydroxyl radicals generated in the lecithin/lipoxidase system (Guo *et al*., 1996); by penetrating the lipid bilayer influencing antioxidant capability in biomembranes as well as reducing the mobility of free radicals into the lipid bilayer (Saija *et al*., 1995, Ostrowska *et al.,* 2004). Moreover, tea can interact with phospholipid head groups, particularly with those containing hydroxyl groups, thus decreasing the fluidity in the polar surface of phospholipid bilayer (Chen *et al.,* 2002). Considerably higher albumin and total protein levels observed in

tea pretreated groups as compared to $CdCl_2$ treated group, indicated that one of the mechanisms by which tea exhibits its protective effect during $CdCl_2$ toxicity is by enhancing the levels of albumin and thereby total protein levels. These observations are in agreement with what was observed by Issabeagloo *et al.,* (2012) who observed a stabilization of the functional enzymes including total proteins and albumin. He attributed this to the ability of tea to prevent intracellular enzyme leakage resulting from cell membrane stability or cellular regeneration.

Heavy metals modify gene expression by interfering with signal transduction pathways that play very essential roles in cell growth and development, resulting to cancer phenotype (Valko *et al.,* 2006). These metals deregulate cell proliferation by activating various transcription factors that are known to control cell cycle progression and apoptosis (Evan and Vousden, 2001). This was evident in this study where ZHX1, a transcription factor was shown to have been reduced by cadmium. This study reports for the first time that the aqueous tea extracts used, protected this transcription factor and significantly increased it to levels comparable with those of the control group that received water only. Although the exact mechanisms on how tea imparted its actions on ZHX1 are not well established, it can be hypothesized that the tea extracts were able to scavenge the free radicals (Rietveld *et al.,* 2003; Karori *et al.,* 2007; Karori *et al.,* 2014), hence reducing oxidative stress and preventing cell damage consequently boosting the endogenous antioxidant capacity. Secondly, tea rescues alpha-tocopherol (Mahmoud *et al.,* 2012), chelates iron and copper, inhibiting further generation of hydroxyl radicals and degradation of lipid hydroperoxides (Azram *et al.,* 2004). Therefore, the chelating action may have prevented ZHX1 from isostructural substitution, replacement with altered geometry, mixed complex formation or catalysis of thiol oxidation by $CdCl_2$ with subsequent heterodimirization of ZHX1 (Kawata *et al.,* 2003). This process would be important in activating transcription of CDC25C: allowing progression of cell cycle from G2 to M phase (Yamada *et al.,* 2003). Further hypothesis may be drawn as follows; that the ability of tea to chelate $CdCl_2$ may allow the HDs of ZHX1 to interact with DNA (cytosine-5-)-methyltransferase 3 beta (DNMT3B), which adds methyl groups to DNA and and enhances its repressive activity (Yamada *et al.,* 2002; Karp, 2005; Kim *et al.,* 2007;Hanahan *et al.,* 2011). However, more research is required in order to elucidate how tea participates in this process.

Furthermore, tea supplementation reduced the elevated GSH levels to near normal levels with those of the control rats that received water only. This would be attributed to the suppression of lipid peroxidation and protein oxidation (Seven *et al.,* 2004). Moreover, it can be postulated that the tea extracts decreased $CdCl_2$ lipophilicity (Mehana *et al.,* 2010), and acted as an alternative sulphydryl nucleophile to GSH (Oda*et al.,* 2010).

Likewise, black and green tea extracts significantly reduced $CdCl_2$ levels in the liver, kidney, bone and testes. This could be ascribed primarily to the presence of specific functional groups in the flavonol structure that endows tea with the ability to directly chelate metal ions (Bilto *et al*., 2012; Hyung *et al.,* 2013).

Histopathology findings in the liver indicated that tea enabled a significant regaining of the normal organization and architecture of the hepatocytes with mild degeneration, suggesting that tea was a potent hepatoprotective compound against Cd induced toxicity (Kumamoto *et al.,* 2001; Chen *et al.,* 2002; ; Azram *et al.,* 2004; Almurshed *et al.,* 2006; Sengottuvelu *et al.,* 2008; Mahmoud *et al.,* 2012)

The hepatic histoarchitecture of the $CdCl_2$-treated rats resulted in severe necrotic changes, inflammatory cell infiltration, fatty degeneration and vacuolization. This might be due to the formation of highly reactive radicals and subsequent lipid peroxidation induced by $CdCl_2$. The accumulated hydroperoxides can cause cytotoxicity, which is associated with the peroxidation of membrane phospholipids by lipid hydroperoxides, the basis for hepatocellular damage. The necrotic conditions coincide with our biochemical observations, which showed the increased level of lipid peroxidation. Administration of black or green tea extracts significantly reduced the histological alterations provoked by $CdCl_2$. This can be attributed to the antiradical/ antioxidant and metal-chelating efficacy of tea which significantly reduced the oxidative stress leading to the reduction of histopathological alterations and restoration of normal physiological state of the rats. Taken together these findings indicate that the administration of tea in cadmium-intoxicated animals counteracted the oxidative hepatic dysfunction attributed to cadmium. Treatment with tea considerably reduced the abnormal changes induced by the metal and restored the biomarkers value of oxidative stress and hepatotoxicity towards near normal. This was sustained by the restoration in the levels of hepatic serum marker enzymes, increase in the activities of antioxidant enzyme cascade, improvement in the levels of non-enzymatic antioxidants along with the decreasing levels of malondialdehyde in the liver. Hepatoprotective nature of tea against cadmium was further supported by the improvement in the histopathological changes occasioned by cadmium. Based on the data generated in this study, it can be concluded that tea played a role of an antioxidant which includes free radical scavenging and metal-chelating property and thereby improved the detrimental state of liver cells which unraveled its use as a possible mitigator/attenuating agent in cadmium induced hepatotoxicity.

4.11.4 Protective effects of aqueous extracts black and green tea on cadmium induced brain toxicity in rats

Cadmium exposure is an recognized occurrence, whose successful treatments are still far from being realized. In view of the human exposure to Cd and its persistent dangers due to its prevalence in the environment and the absence of decomposable processes (Wright and Baccarelli, 2007), there is a strong impetus to develop effective therapeutic strategies for managing its toxicity. While there is no agreement on the effectiveness of chelation therapy for Cd poisoning, some scholars hold the view that chelation therapy may well increase its removal, thus reducing its toxic effects (Saljooghi and Fatemi, 2010; Jamilaldin Fatemi *et al*., 2011). Furthermore, the combination of chelating agents and antioxidants has better effects compared to treatments that use each molecule independently (Gil *et al*., 2011).

Tea is powerful dual antioxidant with metal chelating properties owing to its structural advantage of having numerous hydroxyl groups (Umeno *et al*., 2016). It has been shown to have neuroprotective effects in a number of neuropathological conditions linked to excitotoxicity and oxidative stress (Weinreb *et al*., 2009). There are significant beneficial effects of tea against Cd neurotoxicity (Mandel *et al*., 2004; Sethi *et al*., 2009; Abib *et al*., 2010; Abib *et al*., 2011). However, to the best of our knowledge, this is the first study to compare the protective effects of polyphenol rich tea (*Camellia sinensis*) and EDTA against Cd^{2+}induced neurotoxicity.

The damaging consequences of Cd exposure on the biological antioxidant defense system in the brain and its possible mechanisms as well as the protective effect of EDTA have been studied (Mikirova *et al*., 2011). In the current study, the postulation that tea, a natural product, offers more or comparable protective effects to that provided by the synthetic chelator $CaNa_2EDTA$ in reversing $CdCl_2$ induced neurotoxicity was explored.

Results from this study indicate that the levels of GSH and malondialdehyde (MDA) in the brain were significantly increased following Cd exposure. The increase in GSH was thought to be a compensatory mechanism that is meant to counteract Cd induced oxidative stress (Antonio *et al*., 2003; Sato *et al*., 2011). This is achieved by maintaining a high concentration of GSH in the cells through synthesis (Martin *et al*., 2008). The finding that MDA was significantly increased in the brain following Cd exposure corroborates previous findings from other studies and is linked to the high amount of polyunsaturated fatty acids in the brain and its high oxygen turnover with concomitant H_2O_2 production (Bultel-Ponce *et al*., 2016). .

In addition, subcutaneous Cd administration resulted to a significant decrease in the levels of ZHX1 in the brain when compared to the control group. Cd replaces zinc in many

biological systems (Jaishankar *et al*., 2014) due to their comparable oxidation states, and therefore the decrease observed in ZHX1 in the brain can be attributed to the substitution of Zn^{2+} for Cd^{2+} in the zinc finger motif with subsequent degradation of the mutant protein via the ubiquitin proteasome pathway (Chen *et al*., 2013). Consequently, dysfunctions in ZHX family members, and especially ZHX1 whose expression is slightly higher in the brain, results in the development and progression of neurodegenerative disorders observed in the current study as damage of the cerebral cortex manifested by a marked presence of lymphocytic inflammatory changes and brain necrosis.

Moreover Cd enhanced GFAP that was characterized by round shaped cell bodies and relatively small number of fibrous processes implying astroglial activation and gliosis. This severe activation of astrocytes is associated with an ongoing neuroinflammatory response and neurodegenerative processes in the brain as shown by other studies (Kovalchuk *et al.,* 2015), with morphological alterations in GFAP-expressing astrocytes (McCall *et al.,* 1996).

In line with my hypothesis, my findings established that oral administration of aquoeus black tea extracts (BTE) and aquoeus green tea extracts (GTE) significantly alleviated the symptoms of Cd toxicity in the brain of rats used. The tea extracts significantly reduced the severe brain injuries manifested by significant increases in GSH, MDA and GFAP expression induced by Cd as well as decreases in ZHX1 to levels that were comparable to those of the control rats. The tea extracts also reduced the marked presence of lymphocytic inflammatory changes and brain necrosis. These observations show that BTE and GTE have the ability of maintaining the endogenous antioxidants by scavenging the ROS-induced by cadmium. Based on biochemical and pharmacological studies, I hypothesize that the mechanisms of preventing and modulating cellular redox state due to Cd induced toxicity in the brain by the tea extracts maybe through their ability to restore the activity of antioxidant enzymes, reduction of free radicals generation,terminating the initiation and propagation of lipid peroxidation, metal chelation and through activation of redox sensitive transcription factors and antioxidant enzymes (Higdon and Frei., 2003; Mohamadin *et al.,* 2005).

Consistent with these findings, previous studies have reported the neuroprotective effects of tea against neuronal damage (Lee *et al.,* 2003) by reducing lipid peroxidation in the brain of rats (Etus *et al.,* 2003). The ability of green tea catechins to act as antioxidants has been shown to be linked to its metal chelating capabilities and their potential of reducing oxidative stress (Tournaire *et al.,* 1993), inhibition of peroxynitrite-induced oxidation of dopamine and nitration of tyrosine residues (Kerry and Rice-Evans, 1999). Green tea catechins

which are water soluble antioxidants may chelate iron and copper, thus reducing oxidative stress and chelating free form of iron. Furthermore, it has been shown that consumption of tea prevents nigral damage induced by xenobiotics (Levites *et al.*, 2001; Levites *et al.*, 2002).

On the other hand, the protective effects of black tea extract (BTE) in reducing Cd toxicity could be attributed to the ability of theaflavins to reduce lipid peroxidation (Tandon *et al*., 2002) as well as in stabilizing the integrity of the cell membrane and keeping it intact (Waisberg *et al*., 2003).

It has been demonstrated that for flavonoids to modulate the effects of xenobiotics in the brain, they must have the ability of crossing the blood brain barrier (BBB) which controls entry of such molecules into the brain (Abbott *et al.,* 2002). Results from this study suggest that the protective effects of the orally administered tea extracts could be due to their ability of crossing the blood brain barrier. This is consistent with previous studies that have demonstrated that tea polyphenols are found in the brain after their oral administration (Abd El Mohsen *et al.,* 2002; Rashid et *al*., 2014). This capability of tea polyphenols to cross the BBB and localization in the brain makes it a better candidate for direct neuroprotective and neuromodulatory actions.

A very outstanding observation in the current study was that, the protective effects of both the BTE and green tea extract (GTE) from cultivar TRFK 6/8 were not significantly different. These may be explained by the fact that flavonols are less affected by tea processing and are present in almost comparable amounts in both green and black teas processed from polyphenol rich cultivars (Hossain *et al*., 2004). Theaflavins present in black tea possess almost the same antioxidant potency as catechins present in green tea, and the conversion of catechins to theaflavins during fermentation in making black tea does not significantly alter their free radical-scavenging activity (Shukla *et al*., 1996).

In contrast though, my results demonstrate clearly that both tea extracts out competed EDTA which is a synthetic chelating antioxidant of great repute in protecting the brain against Cd induced damage. This would be attributed to the inefficiency of EDTA as an antioxidant as well as its relatively poor chelating properties due to its structure that gives incomplete shielding of Fe^{3+}, forming an open complex (basket complex) that increases the catalytic capacity of Fe^{3+} for generating oxidative stress (Singh *et al*., 1995). Additionally, EDTA is distributed mainly in the extracellular fluids, which limits its capacity to chelate out metals from inside the cells with consequences of redistributing heavy metals from other tissues to the brain (Flora *et al*., 2010).

Thus, the protective effects of tea against Cd induced toxicity in the brain might represent the consequence of either the to its antioxidative and chelating ability. It is reasonable to assume that daily consumption of small doses of polyphenols derived from tea could be an interesting strategy to prevent the deleterious effects of Cd towards the brain.

4.11.5 Protective effects of aqueous extracts black and green tea on cadmium induced testicular toxicity in rats

The current study revealed that tea preserved normal testicular architecture in comparison to the testis of rats that were challenged with cadmium. Consistent with these findings, studies have attributed such improvement against cadmium intoxication to the antioxidant and chelating activities of tea which binds with Cd ions to form an insoluble complex-ionic salt that was used to remove Cd and restrict the interaction of the metal ion with membrane lipids, thus avoiding oxidative damage to membrane lipids and proteins (Paul, 2008). Subsequent studies have reported that compounds having antioxidant activities, such as vitamin C, vitamin E, Zn, selenium and melatonin, have protective properties that prevents both the oxidative stress and damage in the testes caused by cadmium (Santos *et al.*,2004; Faure *et al.,* 2007 ; Acharya *et al.,* 2008; and Burukoglu and Baycu, 2008). Yang *et al.*, (2006) andAl-Attar (2011) also found out that administration of vitamin E protected the testis of mice exposed to heavy metals as evidenced by appearance of normal structures of seminiferous tubules of testis. It is therefore plausible for this study to report for the first time, that Kenyan black and green tea ameliorate the cadmium induced toxicity in the testes of rats.

This study demonstrated that the liver, kidney, bone, testes and brain constitute sensitive targets for inorganic cadmium, the latter contributing to cadmium induced toxicity. Thus, the toxicity mechanisms of this environmental contaminant include affecting of both enzyme catalyzed reactions, non-enzymatic and cellular activations. Data generated from the current study taken together with epidemiological data from other studies agree that increased intake of tea has potential health benefits attributed to its antioxidant and chelating properties (Setiawan *et al.,* 2001; Sueoka *et al.,* 2001 and Zhang *et al.,* 2004)

While this study has shown that tea has insignificant cytotoxic effects, the benefits outweigh the effects and therefore, it is suggested that tea may be beneficial in alleviating undesirable effects of heavy metal toxicity. It further provides biological insight and backs the usefulness of tea against cadmium induced toxicity and oxidative stress on the tissues that were studied. This data correspondingly supports the hypothesis that both green and black tea aqueous extracts may have beneficial health effects against risks of heavy metal induced

toxicity. It can further be inferred that tea is a better therapeutic agent than the conventional chelating agents in use such as EDTA.

CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

i) Results from this study also revealed that the total catechins levels were statistically different between the two types of tea that were used. Black (aerated) teas had lower catechin levels than the green (non-aerated) teas. Likewise, individual catechins varied significantly among the two types of tea with EGCG, GC and EGC levels being the highest and +C, ECG and EC being less abundant. Furthermore, the levels of theaflavins and thearubigins which are the main products of fermentation increased in black tea when compared to green tea. Additionally, the two types of processed tea leaf used in this study contained some amount of metals. Aluminum was the most predominant and magnesium was the lowest. The study showed that tea is a good source of manganese, copper and calcium in diet as their levels match the acceptable daily intakes and do not therefore expose the tea user to high levels which can be toxic. The nonessential elements including aluminum, lead and cadmium were in very low concentrations in tea infusions and thus do not pose any risks in terms of toxic metals in diet, with their contents matching permissible levels for toxic metals in food and beverages.

ii) Chelation therapy using tea is an efficient strategy that can be employed in the management of heavy metal induced toxicity and restoring the altered biochemical function in the biological system. Data generated from the current study revealed that subcutaneous administration of cadmium chloride significantly induced the renal, hepatic, neuronal, and testicular and bone damage which was evident by increased levels of serum AST, ALT, ALP as well as decreased levels of total proteins, albumin, ZHX1 in brain and liver tissue homogenates. There were also significant increases in lipid peroxidation markers (Thiobarbituric Acid Reactive Substances), reduced glutathione and increased levels of cadmium in the liver, kidney, testes and bones

iii) Co-administration of aqueous black or green tea extracts along with $CdCl_2$ resulted in a reversal of Cd-induced biochemical changes in liver and brain accompanied by a significant decrease in lipid peroxidation and an increase in the level of hepatic, neuronal, testicular and renal antioxidant defense system. There was also a significant reduction in cadmium chloride levels in the liver, kidney, testes and bone tissues. The histopathological studies in the brain, liver, kidney and testes of rats also showed that

the aqueous extracts of black and green tea significantly reduced toxicity of Cd and preserved the normal histological architecture of the tissues examined. This study attributes the cytoprotective potential of tea in Cd toxicity to its antioxidant and metal chelating properties that endow it with the ability of accomplishing its optimum effects.

iv) Data generated from the current study indicates that tea is non toxic and can therefore be a lead compound for new drug discovery against the various putative molecular targets. Rational design of analogs from tea polyphenols would be valuable for structure-activity relationship studies to determine the contribution of the various phenolic groups to the antioxidant-chelating activity and the overall therapeutic effects of green tea and black tea.Tea proved to be a more potent chelating antioxidant than EDTA. Furthermore, this data provides novel insights on mechanisms of cadmium toxicity and may possibly give a lead to tea product diversification in new regimens or pharmacological interventions against heavy metal toxicity.

5.2 Recommendations

i) Diversification of tea to come up with supplements should be pursued as an alternative therapeutic strategy in the management of cadmium induced toxicity.

ii) More studies should be carried out using the extracted individual tea biomolecules to ascertain their mechanisms effecacy in metal toxicity compared to the crude aqueous tea extracts

iii) More studies should be carried out on aqueous tea extracts to ascertain the mechanisms that lead to anemia during their administration.

iv) The currently used metal chelating agents need to be replaced with aqueous tea extracts due to their established side effects and to increase treatment efficacy

v) Future research should focus on larger scale-longer period studies to gain more data on the biochemical, immunohistochemical and histopathological changes in the biological system before and after chelation therapy.

vi) Future research should also be geared towards determination of the most appropriate dosage that will efficiently ameliorate the effects of the heavy metal toxicity.

vii) Consumption of black and green tea extracts should be encouraged as a prophylactic against heavy metal toxicity.

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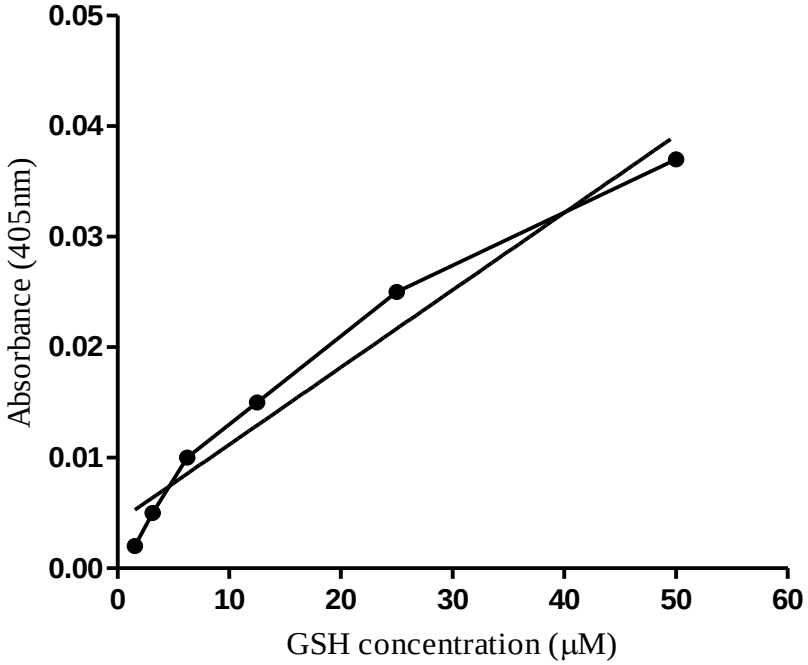
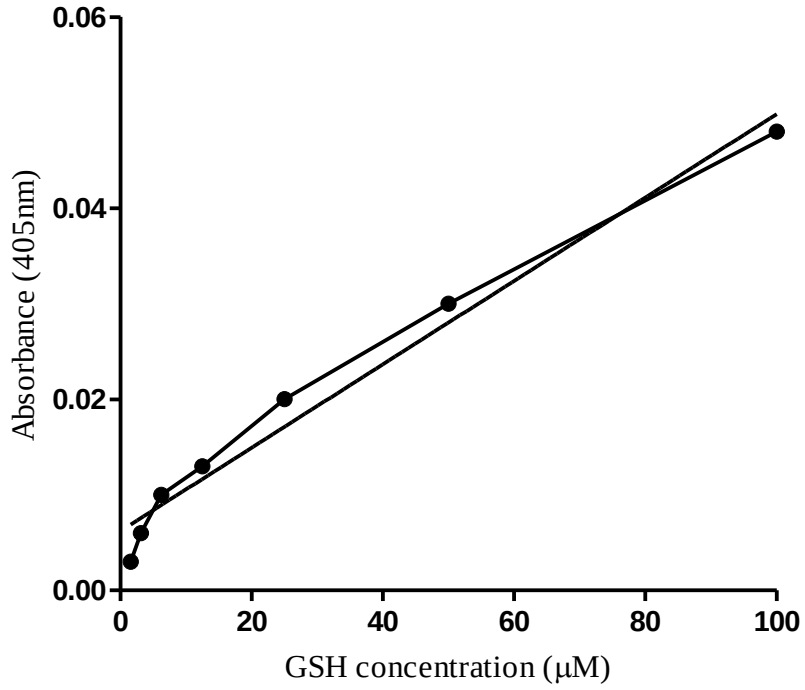
APPENDICES

Appendix I: Total GSH calibration curve -1



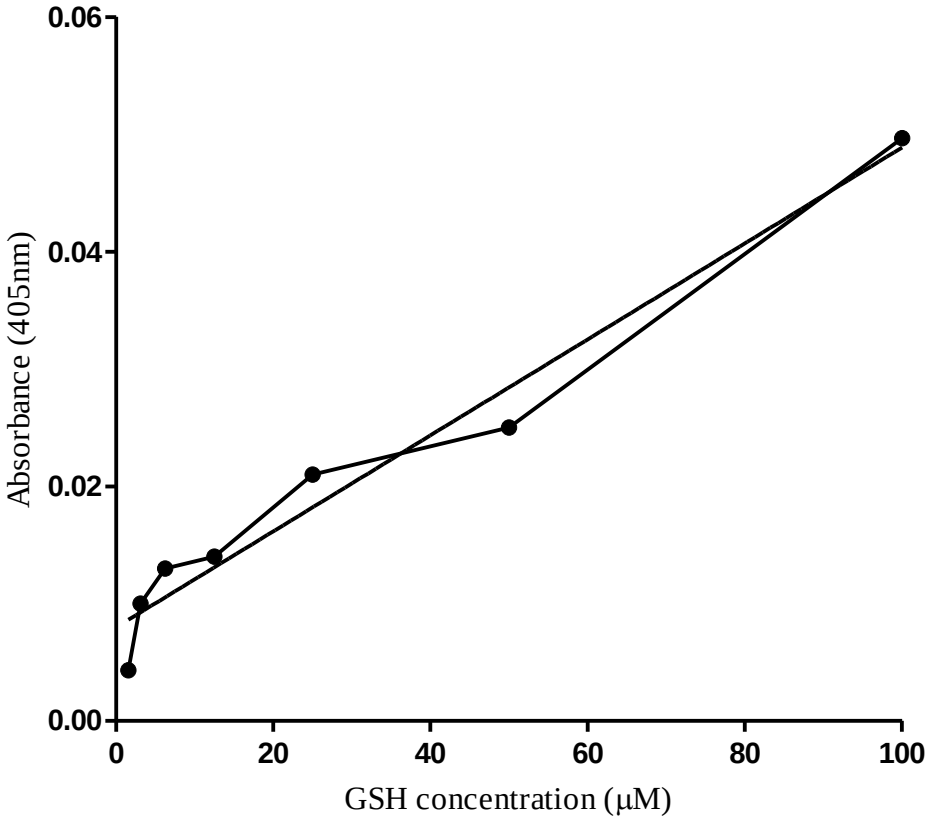
Goodness of fit r^2= 0.9603
Brain data plate 1

Appendix II: Total GSH calibration curve -2



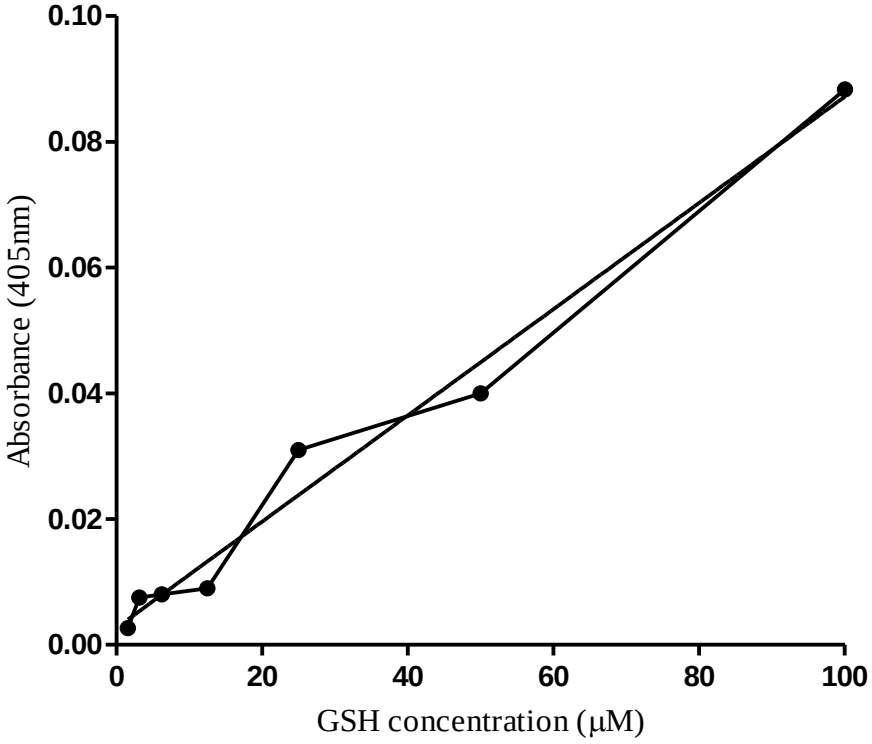
Goodness of fit r^2= 0.9760
Brain data plate 2

Appendix III: Total GSH calibration curve -3



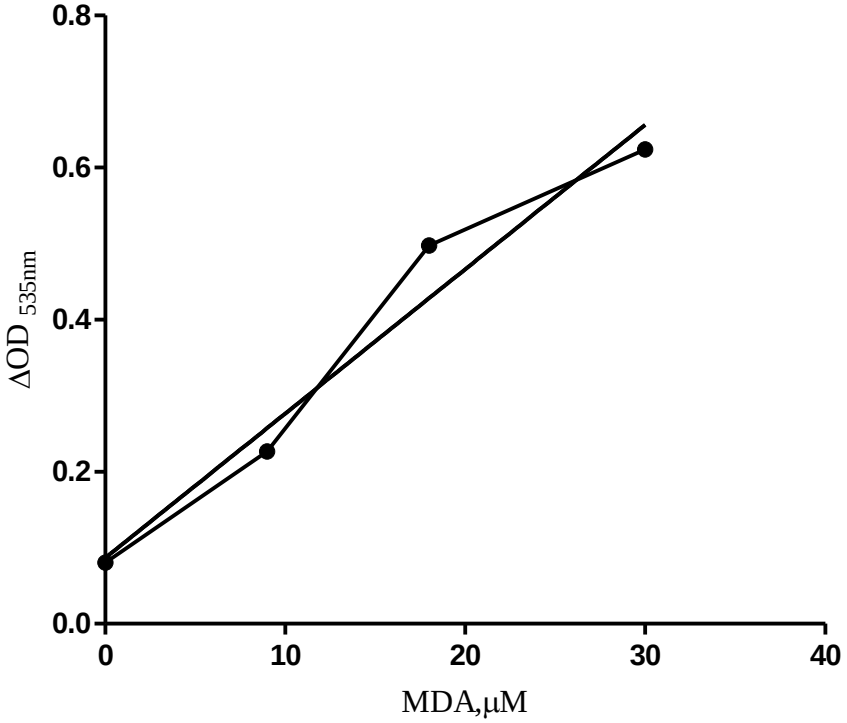
Goodness of fit r^2= 0.9656
Liver data plate 1

Appendix IV: Total GSH calibration curve -4



Goodness of fit r^2= 0.9818
Liver data plate 2

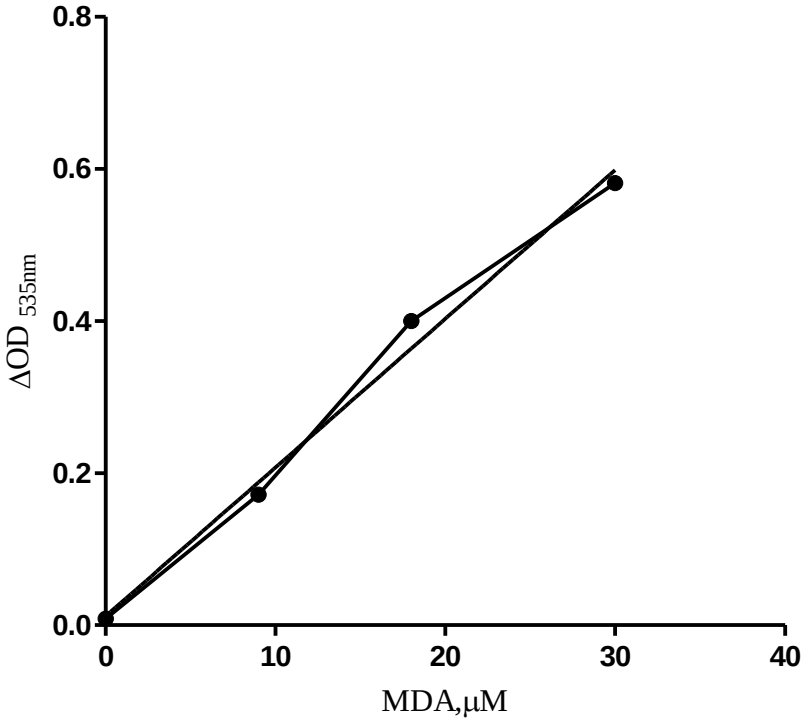
Appendix V: Brain TBARS calibration curve



Goodness of fit r^2= 0.9631

BRAIN TBARS

Appendix VI: Liver TBARS calibration curve



Goodness of fit r^2= 0.9902
LIVER TBARS Plate 1

Appendix VII: Institutional Animal Use and Care Approval Letter

NATIONAL MUSEUMS OF KENYA
WHERE HERITAGE LIVES ON

Institute of Primate Research
Address: P. O. Box 24481-00502 Karen Nairobi Kenya | Tel: +254 02 2606235 | Fax: +254 02 2606231
URL: www.primateresearch.org | Email: directoripr@primateresearch.org

INSTITUTIONAL REVIEW COMMITTEE (IRC)

FINAL PROPOSAL APPROVAL FORM

Our ref: IRC/ 08/13

Dear **Dr. John Wanyoko,**

It is my pleasure to inform you that your proposal entitled "Antioxidant and Chelating Properties of Tea Polyphenols in Ameliorating Cadmium and manganese Induced Toxicity in rats" has been reviewed by the Institutional Review Committee (IRC). The proposal was reviewed on the scientific merit and ethical considerations on the use of animals for research purposes. The committee is guided by the institutional guidelines (e.g. S.O.Ps) as well as International regulations, including those of WHO, NIH, PVEN and Helsinki Convention on the humane treatment of animals for scientific purposes and GLP.

This proposal has been approved and you are bound by the IPR intellectual Property Policy.

Signed..... Chairman IRC. Dr. Hastings Ozwara

Signed..... Secretary IRC. Dr. NGALLA JILLANI

Date: 3rd SEPTEMBER 2013

INSTITUTE OF PRIMATE RESEARCH
INSTITUTIONAL REVIEW COMMITTEE
P. O. Box 24481-00502 KAREN
NAIROBI - KENYA
APPROVED..03/09/2013.....

Appendix VIII: Journal PublicationI

Print ISSN: 2319-2003 | Online ISSN: 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

DOI: http://dx.doi.org/10.18203/2319-2003.ijbcp20183017

Original Research Article

Antioxidant activity and effects of Kenyan Tea (*Camellia sinensis*) on the liver function and serum biochemistry in male Wistar rats

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ABSTRACT

Background: Tea is a beverage that is most widely consumed worldwide. Studies have shown that oral consumption of tea has health benefits however, there is paucity of data in Kenya detailing the biochemical effects of tea in the liver and elucidation of its mechanism of action.
Methods: The polyphenol composition and antioxidant capacity of tea were determined by HPLC and the Folins Ciocalteu spectrophotometric methods. Metal levels were determined using flame Atomic Absorption Spectrometer (AAS). Aqueous black and green tea extracts were administered to the rats at dosages of 400mg/kg b.w.t. The effect of tea on total blood proteins, Albumin, ZHX1, TBARS, AST, ALP and ALT were determined by spectrophotometric methods. The body weight of each rat was also determined at one week interval.
Results: Total Polyphenols (TP), Total Catechins (TC) and Antioxidant Activity (AA) between the black and green teas were significantly ($P<0.05$) different. Green tea had the highest levels of TP (19.70-26.12%), TC (8.51%-17.60%) and AA (86.65%-94.50%). Tea did not have a significant ($P>0.05$) effect on TP, ALB, ALT, AST, ALP, MDA and ZHX1 in the test animals compared with the controls. This data indicates that green tea is rich in catechins while black tea being rich in Theaflavins (TFs) and Thearubigins (TRs). Both tea products possess essential and non-essential metals well within the maximum permissible concentrations.
Conclusions: Findings from this study indicate both green and black tea aqueous extracts have polyphenols and high antioxidant activity. Administration of the aqueous tea extracts have no toxicological effect on the liver.

Keywords: Biochemical, Liver, Polyphenols, Tea

INTRODUCTION

Tea is a beverage that is most widely consumed worldwide.[1,2] Tea has gained popularity due to its immense pharmacological, antioxidant and heavy metal chelating abilities. This beverage is now increasingly being employed in the management of hypertension, arteriosclerosis, hypoglycemia and hypocholesterolaemia.[3] Additionally, it is hypothesized that the effectiveness of tea to manage most of the disease

Appendix IX: Journal Publication II

Research Article Open Access

Neuroprotective Effects of Tea against Cadmium Toxicity

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ABSTRACT

Background: Cadmium (Cd) is a common pollutant and potential neuro-toxicant to humans. The main treatment for heavy metal toxicity is chelation therapy which is however replete with grave side effects. This study was designed to determine the neuroprotective effects of extracts of the tea beverage on experimentally induced cadmium toxicity in the brain of rats. Cadmium as $CdCl_2$ was administered subcutaneously while tea was given orally.

Methods: Healthy Wister rats were used to study the effects of co-administration of Cd and tea extracts on the brain. Cadmium was injected subcutaneously while tea was administered orally to the rats. Brain tissue from euthanized rats was assayed for Zinc Fingers and Homeoboxes Protein 1 (ZHX1), reduced glutathione (GSH), and lipid peroxidation markers Thiobarbituric Acid Reactive Substances (TBARS). Neurohistochemical and histopathological studies were also carried out on the brain tissues of the rats.

Results: Cadmium significantly induced neuronal damage exhibited by a significant ($p < 0.05$) decrease in ZHX1 in the brain tissue, significant ($p < 0.05$) increase in TBARS, as well as significant ($p < 0.05$) increase in GSH implying an impaired antioxidant defense system. Co-