PROTECTIVE EFFECTS OF *Cleome gynandra* LEAF EXTRACT AGAINST ACUTE MERCURY CHLORIDE-MEDIATED KIDNEY DAMAGE IN MALE WISTAR ALBINO RATS

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN MEDICAL BIOCHEMISTRY AT MOI UNIVERSITY.

DECLARATION

Declaration by the candidate:

This thesis is my original work to the best of my knowledge and has not been presented for a degree in any other university or institution. No part of this thesis may be reproduced without the prior written permission of the author and/or Moi University.

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DEDICATION

This thesis is dedicated to my parents and siblings.

ABSTRACT

Background: Mercury (Hg) is an environmental pollutant that causes toxic effects in humans or animals exposed to it at toxic levels that impair some organs' function, with the highest Hg levels observed in the kidney. Associated adverse health effects such as kidney damage, have been reported in populations living in polluted areas including Artisanal Small-scale Gold Mining (ASGM). In Kenya, Masara and Osiri/Mikei artisanal gold miners in Migori County are affected. A promising approach to protecting tissue damage is the use of phytochemicals.

Objective: To determine the protective effect of *Cleome gynandra* (CG) leaf extract on the kidney of male Wistar albino rats exposed to mercuric chloride.

Methods: The study was an experimental animal study. Through cold maceration using absolute methanol as a solvent, the CG extract was obtained from dried, homogeneously ground leaves. 30 male albino Wistar rats were grouped into groups of five randomly: Group I - vehicle control; group II - 5 mg mercuric chloride ($HgCl_2$)/kg of body weight (bwt) subcutaneous injection on the 7^{th} day; group III & IV - 250 and 500 mg CG extract /kg bwt daily oral administration, respectively, and 5 mg $HgCl_2$/kg bwt subcutaneous injection on the 7^{th} day; group V - 0.5 mg $HgCl_2$/kg bwt daily subcutaneous injection; and group VI - 0.5 mg $HgCl_2$/kg bwt daily subcutaneous injection with daily 200 mg CG extract/kg for 7 days. Anaesthesis was performed 24 hours after $HgCl_2$ administration followed by the obtaining of blood samples through cardiac puncture for biochemical parameters analysis in groups I - IV and kidney samples for histological examination in groups I - VI after sacrificing. The mean concentrations of the biochemical parameters were compared using the One-Way Analysis of Variance (ANOVA) followed by Tukey post hoc test ($p \leq 0.05$).

Results: The variations among the groups I - VI were statistically significant for creatinine (p=0.0002), urea (p=0.0002), and albumin (p=0.0082) concentrations. $HgCl_2$ significantly increased urea (p = 0.021) and creatinine (p = 0.032) in the blood compared to the vehicle control. These changes were not prevented by the CG extract. The difference between the groups receiving 250 and 500 mg CG extract/kg bwt was insignificant for creatinine (p=0.972) and urea (p=0.341). These quantitative observations were supported by histological alterations of the kidneys. Albumin in the blood was lower in the treatment groups compared to controls, only significant in treatment group 2 ($HgCl_2$+500mg CG extract/kg bwt) (p=0.005). Administration of 200 mg CG extract/kg bwt limited tissue damage to a minimum at low $HgCl_2$ concentration.

Conclusion: At a low mercury concentration, CG was nephroprotective. However, pre-treatment with CG extract was ineffective in nephroprotection against damage induced by the high dose of mercury.

Recommendations: The findings of the study to be used in the advancement of the knowledge of CG, the methanolic extract of *Cleome gynandra* should not be used in the management of high mercury concentrations, and 10% dimethyl sulphoxide can be considered as a solvent for plant extracts in experimental animal study designs for acute conditions.

Keywords: *Kidney, renal, mercury chloride, Cleome gynandra, urea, albumin, creatinine, histological.*

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ACKNOWLEDGEMENTS

The successful completion of this thesis was made possible by my supervisors, that is, Dr. Caleb Nyamwange and Prof. Hezron Nyandieka. I am grateful for their guidance and stimulating discussions during proposal development, research, and thesis scripting. I also appreciate the Departments of Biological Sciences and Chemistry and Biochemistry of the University of Eldoret for their support and collaboration. I am also grateful to the Department of Biochemistry and Clinical Chemistry of Moi University led by Dr. Geoffrey K. Maiyoh and the Department of Pathology of Moi University, especially Dr. David Chumba, for the conducive learning environment, assistance, and resources. The achievements of this study would not have also been attainable without the assistance of Mrs. Dinah Sawe and Mr. Kennedy Oyamo. Finally, my profound gratitude goes to my parents, siblings, and friends for their support during my study and research.

ABBREVIATIONS

AMPATH	Academic Model Providing Access to Healthcare
ANOVA	Analysis of Variance
ASGM	Artisanal Small-scale Gold Mining
ATSDR	Agency for Toxic Substances and Disease Registry
BUN	Blood urea nitrogen
bwt	Body weight
CG	*Cleome gynandra*
DMSO	Dimethyl sulphoxide
EDTA	Ethylenediaminetetracetic acid
EPA	Environmental Protection Agency
F	Ratio of two variances (Between-group variance and within-group variance)
g/dL	Gram per deciliter
g/L	Grams per liter
g	Gram
Hg^{2+}	Divalent mercury
$HgCl_2$/g	Mercuric chloride per gram
$HgCl_2$/kg	Mercuric chloride per kilogram
$HgCl_2$	Mercuric chloride
Hg°	Elemental mercury
Hg	Mercury
HPLC	High Performance Liquid Chromatography
Kg	Kilogram

mg/dL	Milligram per deciliter
mg/kg	Milligram per kilogram
mg	Milligram
ml	Milliliter
mmol/L	Millimole per liter
MO	Missouri
mRNA	messenger ribonucleic acid
MTRH	Moi Teaching and Referral Hospital
NRC	National Research Council
n	Sample size
OECD	Organization for Economic Organization and Development
pH	potential for Hydrogen
ROS	Reactive Oxygen Species
rpm	Revolution per minute
rpm	revolutions per minute
SD	Standard deviation
-SH	Sulfhydryl group
UNDP	United Nations Developmental Programme
UNEP	United Nations Environmental Programme
USA	United States of America
W/V	Weight by volume
WHO	World Health Organization
µg/m^3	Microgram per cubic meters
µmol/kg	Micromole per kilogram
µmol/L	Micromole per liter

%	Percent
^{0}C	Degrees Celsius
®	Registered trademark

DEFINITION OF TERMS

Acaricidal: Pesticide property capable of killing mites and ticks.

Antifeedant: Ability to induce feeding deterrence in insects.

Apoptosis: Biochemical alterations leading to cell death.

Atrophy: Reduction in the mass of a tissue or an organ due to cell numbers reduction.

Bioaccumulation: The net contaminant accumulation within a living thing from exogenous sources.

Bioavailable: Quantity of a chemical present in the systemic circulation.

Biomarkers: An objectively measured and evaluated characteristic to indicate normal or abnormal biological functions, or biological response to treatment.

Chelation: a process of administering a medication to remove heavy metals from the blood.

Cryogenic: Conditions of very low temperatures.

Desquamation: Shedding of the epithelial layer.

Divalent: Requires two electrons to have a zero charge.

Fibrosis: Formation of fibrous connective tissue in response to damage or injury.

Glutathione: A tripeptide containing the amino acids glutamic acid, glycine, and cysteine.

Histological or histopathological: Associated with the structure of cells and tissues of organisms.

Hyalinosis: Deposition of hyaline, a fibrillar protein, on the walls of vessels and connective tissue.

Hypoalbuminemia: A biological state characterized by a low concentration of blood albumin.

***In vitro*:** A condition that simulates the living processes of organisms.

***In vivo*:** A condition in which research is carried out within or with a whole living organism.

Lobulation: An appearance resembling a small lobe.

Maceration: An extraction technique for essential oils and active compounds where powdered plant material is kept in contact with the solvent at room temperature.

Mesangial cells: Specialized kidney cells that mainly support the coil and loops of capillaries.

Metallothionein: A binding protein specific to metals.

Necrosis: Uncontrolled cell death characterized by the disintegration of cell compartments.

Nephrotoxic: Toxic to the kidney.

Pars recta: The straight descending portion of the proximal tubule of the kidney.

Photomicrographs: A picture of a microscopic element or object captured with the help of a microscope.

Phytochemicals: Naturally occurring compounds in plants that are non-nutrient and of medical value.

Polyphenol: Secondary metabolites in the plant kingdom composed of one or more phenolic hydroxyl groups.

Pyknosis: The shrinking of the nucleus accompanied by chromatin condensation.

Sclerosis: The hardening of a body part due to the formation of fibrous interstitial tissue.

Selenohydryl: A functional group containing selenium and hydrogen atom.

Sulfhydryl: A reactive and ubiquitous ligand in a biological system composed of sulfur and hydrogen atoms.

Toxicity: Causing a disturbance in the normal functioning of an organ or life process.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Mercury (Hg) is a hazardous environmental pollutant that is bio-accumulative and toxic to human health (ATSDR, 1999; WHO, 2007, 2013). Irrespective of these qualities, it has found applications in various fields due to its unique properties. These fields include the manufacturing industries (Hg cell chlor-alkali battery, barometers, and thermometers production); mining sector; and dentistry (dental amalgam fillings, containing up to 50% elemental mercury) (Budnik & Casteleyn, 2019; Poulin & Gibb, 2008; WHO, 2003). If the emissions and wastes from these sectors are not adequately managed, they can be hazardous. Combustion of coal and incineration of mercury-containing waste products also releases Hg into the environment (UNEP, 2010). Additional to human activities, natural events such as volcanic eruptions also release mercury into the environment (ATSDR, 1999). Although both anthropogenic and natural sources release mercury into the environment, human activities result in mercury contamination of the environment (UNEP, 2010).

Human activities account for half to two-thirds of the total annual mercury release globally. The artisanal and small-scale gold mining (ASGM) sector is among the primary anthropogenic Hg emission sources, accounting for 37% of global emissions (approximately 400 metric tons) (EPA, 2021) due to the use of mercury in gold recovery through the mercury amalgamation process. At the same time, this sector has the greatest demand for mercury, where almost all of the mercury used finds its way into the environment in tailing, as spills, and/ or fumes (EPA, 2021; Gerson *et al*., 2013; UNEP, 2010).

It is estimated that the number of individuals working in the mining sector (ASGM) globally is 14-19 million. Across 70 developing countries, in South America, Sub-

Saharan Africa, and Asia, the number is more than 10 million, with over 3 million women and 1 million children exposed daily to Hg vapour levels exceeding the World Health Organization (WHO) guidelines' tolerable, non-lethal concentration of 0.2 $\mu g/m^3$ (Calao-Ramos *et al*., 2021; Cordy *et al*., 2011; UNEP, 2010; WHO, 2007). In Kenya, the estimation is at 146,000 individuals (Steckling *et al.*, 2017). However, the number could be as high as 250, 000 individuals (UNDP, 2017) while Mitchell *et al.* (2020) estimated a lower workforce of 40, 000 individuals.

Since some of these individuals handle mercury without adequate protection, they tend to be negatively affected. The localization of these effects cannot only be specific to the mining site since mercury vapour can be airborne and regions without or with negligible mercury emissions can also have high readings of mercury vapour (Poulin & Gibb, 2008). Intending to curb mercury's usage negative impacts, as a state, Kenya is part of the Minamata Convention on Mercury, where it has pledged to limit mercury use, transition the ASGM sector from a subsistence or informal level into a formal level, encourage ethical practices, and limit health issues faced by the mining communities (Mitchell *et al.*, 2020).

Exposure to metallic mercury (Hg°) in the ASGM mining regions leads to its increased presence in the body as shown by the high mercury levels in the blood (above 5 ng/mL), urine (above 100µg/L), and hair (above 1 parts per million) (Bell *et al*., 2017; Steckling *et al.*, 2014). Elemental mercury (Hg°) is majorly taken in the form of mercury vapour, absorbed rapidly and efficiently (70-80%) through the alveolar membrane. Through the gastrointestinal tract and skin, absorption of Hg° is low with negligible retention by the tissues (Nordberg *et al*., 2005). The tolerable, non-lethal concentration is 0.2 $\mu g/m^3$, for prolonged exposure (WHO, 2007). After absorption, it rapidly diffuses into the blood. Also, it readily permeates the blood-brain barrier. The

absorbed Hg° distribution resembles many aspects of the distribution of inorganic mercuric salts because Hg° is rapidly oxidized to mercuric ion (Hg^{2+}) in biological fluids (Zalups, 2010). This oxidative process occurs in the red blood cells and tissues, a process attributed to catalase oxidation (Zalups, 2010). With time, after exposure, mercury deposit is majorly found in the kidney, though deposition also can occur primarily in the brain (Park & Zheng, 2012).

In the body, it induces toxic effects impairing functions of organs and organ systems like the kidney, liver, and developing brain (Nordberg *et al.*, 2005). The impairment depends on mercuric ions' ability to bind to sulfhydryl and selenohydryl groups of peptides due to their electrophilic nature. As a result, their structure is altered and their function is inhibited (Bernhoft, 2012; Spiller, 2018). Faecal matter and urine are the key excretion pathways, although saliva, sweat, and breath eliminate small quantities of inhaled mercury (Park & Zheng, 2012).

In cases of an acute exposure, due to the poor absorption rate in the gastrointestinal tract, the toxicological effects are not significant. On the skin, acute exposure to Hg° can cause dermatitis; and subcutaneous granuloma as a result of self-injection (Park & Zheng, 2012). Poisoning through inhalation of mercury vapour damages the lungs severely and possibly cause death. Such exposures are often accidental, especially in enclosed and high-temperature settings in industries or even at home. In a majority of cases, the inhalation negatively impacts the central nervous system evidenced by memory loss, ataxia, tremors, and increased sensitivity to stimulation, which are often reversible (Saturday, 2018).

Chronic exposure mainly affects the central nervous system while acute exposure affects the lungs and the kidney. Major symptoms include tremors, erethism, and psychological issues. In the lungs, features such as pulmonary oedema and capillary

damage are evident as a result of mercury absorption. The changes in the kidney range from inflammation to necrosis in the tubules and glomerulus (Asano *et al.*, 2000). The tubular and glomerular damage is commonly signified by proteinuria (Park & Zheng, 2012). Cases of hepatic and neurological dysfunction also exist after mercury exposure (Asano *et al*., 2000)

The highest levels of mercuric ions have been observed in the kidney (34.82 ug/g of wet tissue at 1.0 mg $HgCl_2$/kg/day) compared to the brain (no mercury was detected) and liver (6.34 ug/g of wet tissue at 1.0 mg $HgCl_2$/kg/day) (Hussain *et al.*, 1999), where it affects renal function and can cause renal failure (Nordberg *et al.*, 2005). Its toxic health effects are exerted through mechanisms like; oxidative stress and lipid peroxidation (altering membrane integrity), altering intracellular calcium ion or distribution, and protein function inhibition (Zalups, 2000).

From blood circulation, through the transport function of organic anion transport, 1 and 3 (Oat1 & Oat3) in the proximal tubule, Hg is taken up into the kidney. Since the mercuric ion binds strongly with thiol-containing enzymes, the irreversible oxidation of the enzyme inactivates them through the thiol group (Rana *et al*., 2018). Often the effect is the unavailability of reduced thiol groups (thiol content depletion) accompanied by oxidative stress. Inactivation of thiol-containing proteins, as in the case of the sodium-potassium pump, interrupts the membrane potential affecting the integrity of cells and the size of the cells and their organelles. Interestingly, Hg metabolism increases sensitivity to apoptosis influenced by Nuclear Factor- kappaB that plays a role in apoptosis hence renal damage (Rana *et al*., 2018).

Several approaches have been proposed and applied in managing the toxic effects of divalent mercury. One is limiting further exposure by removing an individual from the contaminated source or environment and washing exposed surfaces (e.g. skin, eyes, and

clothing) with water or normal saline, and the washings managed as hazardous waste. Secondly, through limiting absorption by oral administration of protein solutions or sulfhydryl-containing molecules. The third is reducing the body's burden by chelation therapy and induced perspiration. Lastly, by interfering with its mechanism of action, using antioxidants and inorganic chemicals like selenium (ATSDR, 1999; Bernhoft, 2012; Rafati-rahimzadeh *et al.*, 2014).

Boosting or supplementing the defence system of the body with exogenous phytochemicals is a promising approach to managing tissue damage (Kasote *et al*., 2015). In this connection, non-enzymatic compounds (Agarwal *et al*., 2010a; Agarwal *et al*., 2010b; Nava *et al*., 2000; Özer & Ayanogˇlu-Dülger, 2003; Pereira *et al*., 2018; García-Niño & Pedraza-Chaverrí, 2014) and plant extracts (Abarikwu *et al*., 2017; Alam, 2007; Oda & EL-Ashmawy, 2012) have been shown to have beneficial effects against mercury related toxicities. Although many plants exhibit substantial beneficial properties in *in vitro* assays, *in vivo* investigations are limited (Chand & Dave, 2014). The *in vitro* assay findings cannot be extrapolated to the *in vivo* assay due to the physio-pharmacological processes of absorption, distribution, metabolism, storage, and excretion, which may affect their *in vivo* therapeutic efficacy (Badarinath *et al*., 2010). *In vivo* assays may be useful in ascertaining the therapeutic application of phytochemicals.

Cleome gynandra L., commonly known as Spider plant or cat whiskers, is an indigenous African and Asian leafy vegetable of the family Cleomaceae (figure 1) (Omondi *et al*., 2017). It is majorly cultivated in the tropics and subtropic regions, especially in areas receiving low rainfall (Moyo & Aremu, 2022). In various African nations, the leafy vegetable is an essential food in the rural setting. In Kenya, local communities have different names for it: dek (Luo), isakiat (Turkana), chinsaga (Kisii),

thageti (Kikuyu), saget (Kalenjin) and Tsisaka (Luhya) (Onyango *et al*., 2013). Increasing evidence from experimental and epidemiological studies of *C. gynandra* have shown some nutritional aspects and identified compounds of potent therapeutic importance (Adhikari & Paul, 2018). Hence, its increased recognition among leafy vegetables as a potential source of nutrients.

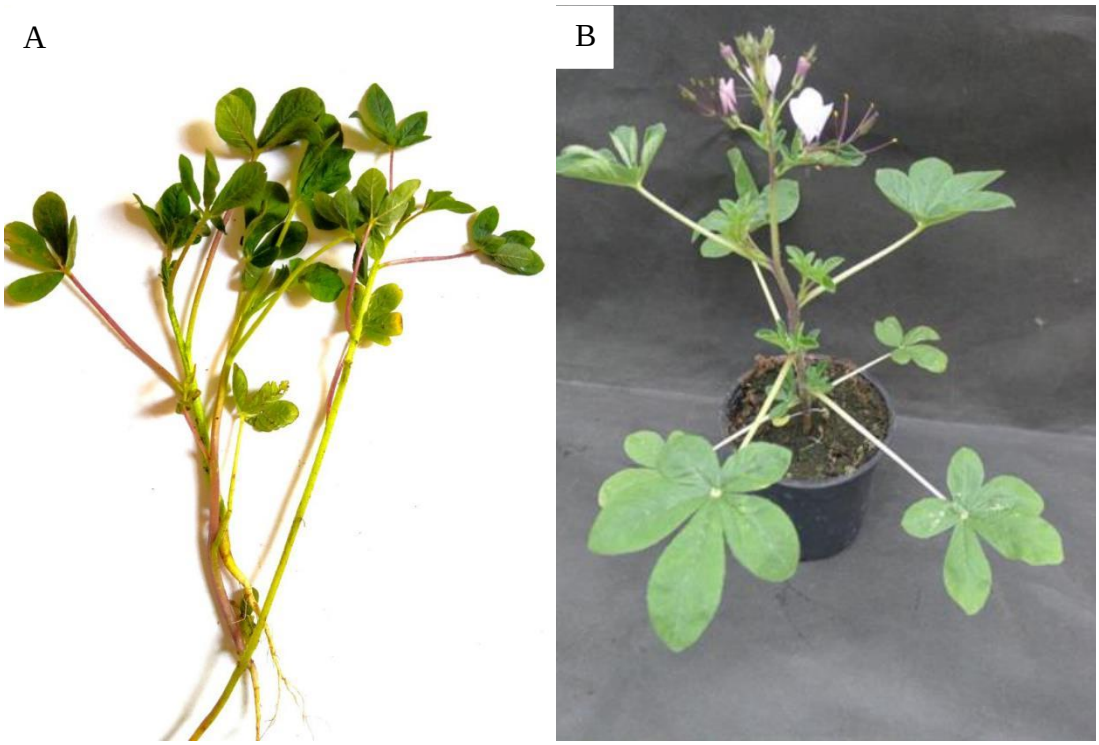

Figure 1: **A -** A harvested cultivate *Cleome gynandra plant;* **B -** *Cleome gynandra* plant under green house conditions (Omondi *et al*., 2017)

Different parts of the spider plant have beneficial health effects, where leaves, young shoots, and in some cases, roots, are consumed. Leaves may be crushed to produce a concoction, boiled and marinated in sour milk, or boiled in water (Van den Heever & Venter, 2006). Indigenous information about its usage as traditional medicine has been reported. *C. gynandra's* application in reproductive health is evident in literature focusing on ethnobotany. Traditionally, leaves and roots have been used by women

before and after parturition to enable them to deliver efficiently and promote the production of milk and its secretion, respectively. It is believed that its consumption reduces the duration of labour, making recovery quick (Van den Heever & Venter, 2006; Onyango *et al*., 2013). Additionally, after childbirth, it can promote the removal of the placenta and manage bleeding, especially, the seed powder (Kamatenesi-Mugisha & Oryem-Origa, 2007). Since in remote rural areas most expectant women deliver at home, its usage is valuable. The leaves have been used in the treatment and management of pain in the inner or middle ear (otalgia), scurvy due to its vitamin C content, epileptic fits, diarrhoea, and constipation (Van den Heever & Venter, 2006; Onyango *et al*., 2013). For chest pains, a root infusion has been used. It has also found applications in healing bacterial infections, headaches, and snakebites (Omondi *et al*., 2017). These traditional applications hint at the presence of secondary plant metabolites beneficial to health.

In addition to traditional applications, some therapeutic investigations by various scholars exist. The leaf infusion has been applied as an eyewash and the leaf sap is used as a pain-relieving agent (Adhikari & Paul, 2018). The management of pain-related conditions indicates that it contains anti-inflammatory phytochemicals which can manage inflammation. Some studies have shown that the spider plant has anti-carcinogenic properties in a dose-dependent manner in tumour-bearing mice (Bala *et al.*, 2010), anti-oxidizing properties in arthritic rats where free radical scavenging ability was confirmed (Muchuweti et al., 2007) and anti-inflammatory properties (Narendhirakannan *et al*., 2007). In managing malaria, *C. gynandra* leaves decoction is used. Among the medicinal plant foods, it is considered beneficial in the management of the effects of malaria which is widespread in sub-Saharan Africa (Moyo & Aremu, 2022). Management of anaemia with *C. gynandra* could be indicative of the rich iron

content in its leaves (Ahouansinkpo *et al.,* 2016). Iron deficiency anaemia is a common anaemic condition brought about by iron's inadequate intake in the diet, leading to reduced haemoglobin and red blood cell count (Ku *et al.,* 2017). It also exhibited nephroprotection in streptozotonin-induced diabetic results, as indicated by the creatinine and urea content (Narsimhulu *et al.,* 2019).

C. gynandra has also been observed to exhibit repellent, antifeedant, and insecticidal characteristics. The leaves can repel and kill ticks. The acaricidal and repellent properties are specific to larvae, nymphs, and adult *Amblyomma variegatum* and *Rhipicephalus appendiculatus* ticks. Ethanol extracts are harmful to diamondback moths and painted bugs infesting cruciferous vegetables. Its antifeedant property is observed against tobacco caterpillars (Van den Heever & Venter, 2006).

Phytochemical investigations have shown *C. gynandra* to be rich in nutrients like vitamins, proteins, and lipids (Ekpong, 2009). Table 12 is a presents the phytochemicals and minerals present on *C. gynandra* and figure 13 is an example of phytochemical screening of *C. gynandra*. Vitamins observed include carotenoids (pro-vitamin A), vitamins C, and E (Gowele *et al.*, 2019). Examples of carotenoids found in the spider plant include lutein, beta-carotene, alpha-carotene, beta-cryptoxanthin, and violaxanthin (Moyo & Aremu, 2022). Carotenoids are essential to the immune system and limit development of certain diseases (Baiano & Del Nobile, 2019). Vitamin E types present in the leaves of the spider plant include alpha-tocopherol, beta-tocopherol, and gamma-tocopherol which have antioxidant properties (Moyo & Aremu, 2022), however, their concentration is dependent on environmental conditions, maturity, and harvest factors (Chun *et al*, 2006).

Additionally, other authors have indicated the presence of kaempferol (Lingegowda *et al.,* 2012), saponins, iridoids, gallotannins, free gallic acid (Moyo *et al.,* 2013),

alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenols, sugars, and triterpenes (Adhikari & Paul, 2018). Also, it is rich in minerals, both macro-nutrients and micro-nutrients. The notable macro-nutrients include sulfur, potassium, phosphorous, magnesium, and calcium, while the main micro-nutrients are manganese, zinc, and iron (Moyo & Aremu, 2022). The difference in genotype influence influences the mineral content in the plant (Omondi *et al.*, 2017).

Since Hg toxic health effects involves mechanisms such as affinity for sulfhydryl functional group, oxidative stress induction, and lipid peroxidation, the chemical diversity of plants with antioxidant properties and metal binding molecules can be beneficial in the management of the toxic effects of Hg to the kidney. In an *in vitro* antioxidant screening, an aqueous methanolic CG extract exhibited a high inhibitory effect (Muchuweti et al., 2007). A compound like caffeic acid exhibits good antioxidant activities (Kim et al., 2006). Compared to ferulic acids, flavonoids (present in CG) also have high antioxidant activity due to the presence of hydroxyl, methoxy, or alkyl groups (Ismail et al., 2004; Muchuweti et al., 2007). Also polyphenolic phytochemicals such as flavonoids have an affinity for heavy metals (Hider et al., 2001) like mercury due to the presence of hydroxyl groups. Hence, the body burden of mercury can be reduced (Magdalena & Brzóska, 2018), reducing tissue injury. Since Hg is toxic an animal model of male Wistar albino rats (figure 2) is used to investigate the protective effects of *C. gynandra* to the kidney against mercury exposure.

Figure 2*:* Male Wistar albino rats.

1.2 Problem statement

When poorly handled, mercury(Hg) is harmful to the human health (ATSDR, 1999; UNEP, 2010; WHO, 2003). Adverse health effects associated with Hg toxicity such as kidney damage have been reported in populations living close to mercury polluted areas, for example, Artisanal Small-scale Gold Mining (ASGM) sites where mercury is used to obtain gold from ore in several countries, Kenya included (Drasch *et al*., 2001; Grotto *et al*., 2010; Ogola *et al*., 2002; Steckling *et al*., 2017). In Kenya, Migori county is an example of a key ASGM centre due to the Migori greenstone belt (Mitchell *et al.*, 2020). In these areas, the frequency of exposure is high (Abarikwu *et al*., 2017)**.** The longer the exposure, the greater the harm it has on their health (ATSDR, 1999). The safety of the mining process and effect of mercury on the environment are main concerns to the local ASGM communities (Mitchell *et al.*, 2020).

In reducing the mercury body burden, chelation therapy offers a good remedy. However, effective chelation therapy protocols often require the replacement of essential minerals. It is because not only do chelators have a high affinity for Hg^{2+}, but

also essential divalent cations in the body (Bernhoft, 2012). Simultaneously, the mercuric ions, which are readily chelated, are challenging to eliminate from the body, and they have a body burden half-life of one to two months (ATSDR, 1999). Due to these limitations, focusing on inhibition on the mechanism of action if mercury and chelation is a viable option. Considering that depletion of endogenous thiol content and oxidative stress are involved in the toxicity of inorganic mercurial, phytochemicals can be beneficial in managing mercury poisoning and offer protection against the effects of mercury poisoning (Oda & El-Ashmawy, 2012). Since *Cleome gynandra* has exhibited its beneficial properties in *in vitro* (Kipandula *et al*., 2014; Meda *et al*., 2013) and *in vivo* models (Narendhirakannan *et al*., 2005), the question is, can it offer protection against mercury-mediated kidney damage? This study, therefore, aims to investigate the protective effects of *C. gynandra* extract against mercury-chloride mediated kidney injury. Since Hg is toxic an experimental rat model is used to investigate the protective effects of C. gynandra to the kidney against mercury exposure.

1.3 Justification

Since exposure to toxic levels of mercury (Hg) can cause mild or gross renal damage and acute renal failure (Poulin & Gibb, 2008), this study was undertaken to determine the protection of *Cleome gynandra* extract against high and low mercury doses. Focusing on the kidney is because it is a primary accumulation organ of elemental and inorganic mercury salts and is susceptible to Hg toxicity (Bernhoft, 2012). At a toxic concentration of Hg, irreversible structural and functional changes can be induced on biomolecules (Brieger *et al*., 2012)**.**

Boosting the body's defense system with exogenous phytochemicals from *C. gynandra* extract was the primary choice of the study. The chemical diversity of natural plant products offers opportunities for discovery and development of herbal remedies

(Elliot, 2018). The experimental animal model for this study is the male albino Wistar rat that have low amounts of estrogen which inhibit the expression of organic anion transporters 1 and 3 that play a role in mercuric ion uptake in the kidney (Hazelhoff *et al.*, 2012). As a human condition model, rats have body system functions similar to humans, a size proportionality of critical organ substructures that affects drug administration and can be surgically operated (Ashish, 2015; Iannaccone & Jacob, 2009). This study was therefore designed to investigate the protective effects of the spider plant extract against mercury-induced kidney damage in male albino Wistar rats. In *in vivo* assays, the protective potential of plants is often assessed by evaluating the effects of phytochemicals on tissue damage biomarkers or antioxidant enzymes activities in experimental animals (Kasote *et al*., 2015). In this study, urea, creatinine, and albumin concentrations in the blood, and histological alterations function as indicators of renal injury, primarily indicating decreased glomerular filtration.

1.4 Significance

The results from the study adds new information to the current knowledge of the spider plant's properties in the context of development of plant-derived remedy for management of mercury toxicity. Research is likely to be furthered on the individual compounds contained in the extracts and their mechanisms of action against Hg^{2+} or heavy metal-mediated injuries in the kidney. These considerations can also apply to other affected body organs. For these reasons, it can influence the design of future multi-component botanical remedies and dietary supplements.

1.5 Research question

1. Does *Cleome gynandra* (CG) possess protective effects against kidney damage induced by a high and low dose of mercuric chloride?

1.6 Broad objective of the study

The general objective of the study was to investigate the protective effects of *Cleome gynandra* (CG) leaf extract against acute mercury-induced damage to the kidneys of male albino Wistar rats.

1.6.1 Specific Objectives

1. To determine the protective effect of CG leaf extract on the blood biomarkers of kidney function and histology of the kidneys of albino Wistar rats exposed to a high dose of mercuric chloride per kilogram of body weight.
2. To determine the protective effect of CG leaf extract on the histology of the kidneys of rats exposed to a low, non-lethal dose of mercuric chloride per kilogram body weight.

CHAPTER TWO: LITERATURE REVIEW

2.1. Acute kidney injury

Acute kidney injury (AKI) is a functional kidney condition characterized by a decline in the kidney function in a duration limited to 7 days (Kellum *et al.* 2021). Although the emphasis is mostly on the severe cases of acute decline in the kidney function, relatively mild instances of renal injury or kidney function impairment indicated by minor alterations in serum creatinine and urine production (glomerular filtration rate) can point to a serious clinical consequence (Makris & Spanou, 2016). Often AKI lacks a distinct pathophysiology, where nephrotoxicity, ischaemia, ans sepsis might exist simultaneously (Kellum *et al.* 2021).

2.1.1. Epidemiology

The variations in the incidence of AKI are dependent on the population under focus, geographical area, and definition used (Makris & Spanou, 2016). Globally, in both adults and children, in high-income settings hospital-acquired AKI is predominantly observed, while in lower-income countries, community-acquired AKI is more predominant . Under-reporting of AKI cases is the reason for the insufficiency of the true information of AKI impact in various areas of the world (Makris & Spanou, 2016). The prevalence of AKI in developed nations is on the rise, where in hospitals it is estimated at 15%, but it is more prevalent (60%) in critically ill patients. community-acquired AKI's incidence is estimated at 4.3%, though it is considered an underestimate (Makris & Spanou, 2016). Globally, the mean age of AKI patients is 60 years, however, with a declining socioeconomic status it is decreasing to 50 years. Independent of other factors, men are 60% of AKI patients, pointing to a sex related risk (Bairey Merz *et al.*, 2019; Mehta *et al.*, 2016). However, socioeconomic status accounts for a higher risk of AKI (Kellum *et al.* 2021)**.**

In sub-Saharan Africa AKI burden is not well known, but a high mortality rate is assumed due to the limited access to health care (Kahindo *et al.*, 2022). The epidemiological data is diverse between countries making it challenging to use it as reliable data. The data are mostly generated from urban settings limiting generalization, though they make considerable contributions to the knowledge about AKI burden (Rewa & Bagshaw, 2014). in Sudan, in a period of over one year, the prevalence was 5.7% for all adult admissions in a tertiary hospital **(**Osman *et al.*, 2017). In South Africa, a prevalence of 6.2% was observed in adult populatuion in a Cape Town tertiary hospital. Reporting AKI incidence in intensive care settings, the incidence exceeded 50% of the admissions in Kinshasa, Democratic Republic of Congo (Masewu *et al.*, 2016). In Cameroon, an anuual incidence of 15 per 100 patients in a year has been observed (Halle *et al.*, 2018). During a nine-months study, a prevalence of 1.05 was observed in a tertiary hospital in Nairobi Kenya (Munyu, 2008).

2.1.2. Causes of acute kidney injury

Globally, the causes of AKI in high income countries are treatment procedures or diagnostic or post-surgical interventions. For community-acquired AKI, its causes include toxins, sepsis, and pregnancy (Kellum *et al.* 2021).In sub-Saharan Africa various causes of AKI exists, including: intoxications e.g. traditional treatments and chemicals; venomous bites e.g. insect, spider, and snake bites; infectious disease e.g. Ebola and malaria; and pregnancy or childbirth complications (Kahindo *et al.*, 2022).

One of the toxicants that negatively affects the kidney in mercury in its inorganic form. Acute poisoning with inorganic mercury targets the kidney where the patient can develop renal tubular necrosis. Exposure to inorganic mercury can be due to an occupational hazards (Sharma *et al.*, 2018). Renal injury usually develops within 24 hours and symptoms such as glycosuria and albuminuria are observed. Histologically,

lesions are observed in renal cortex. These observations were made in case study of acute inorganic mercury poisoning (Sharma *et al.*, 2018). In Africa, the rise on kidney damage cases can be linked to the gold rush that uses the highly effective mercury in processing gold (Githiria *et al.*, 2020).

2.2. Use of blood biomarkers in determining kidney function and injury due to mercury toxicity

Blood urea nitrogen (BUN) and creatinine are metabolites (waste products) excreted by the kidney (Tootian *et al*., 2012). Therefore, they are suitable biomarkers for renal damage. Conceptually, acute kidney damage is characterized by decreased renal excretory function, leading to the accumulation of nitrogen metabolites such as creatinine and urea. However, the blood urea level can be dependent on nutrition, metabolism, muscle mass, prerenal influences, race, sex, and age in humans. On the other hand, serum creatinine level is independent of these factors (Liu *et al*., 2016). An increase in serum creatinine level usually follows an increase in serum urea level in the initial stages renal damage (Liu *et al*., 2016).

Mercuric chloride is a harmful nephrotoxin capable of affecting serum parameters associated with kidney function (Gado *et al*., 2014). The renal injury causes abnormalities in the kidney's function and structures that persist for more than 6 hours. Generally, acute kidney injury is characterized by a sudden serum creatinine rise ($\geq$ 0.3 mg/dL or 26.5 µmol/L) in less than 48 hours or one and a half times in seven days (Tootian *et al*., 2012). In an experimental toxicity animal study, a mercuric chloride dose (5 mg/kg of body weight) significantly increases blood urea and serum creatinine as a biochemical manifestation. The biochemical alterations observed are an indication of nephrotoxicity (Gado *et al.*, 2014). Beohm *et al.* (2002) also showed that an increase

in serum creatinine levels was accompanied by a decreased glomerular filtration rate and urine volume.

Subcutaneous injection of 4 mg of mercuric chloride per kg of body weight also has the same effect on serum urea and creatinine (Alam, 2007). At sub-acute (28 days) low doses of mercuric chloride (0.02 mg per kg body weight) the creatinine and urea tend to increase compared to the control group (Gökc *et al*., 2016). In human studies, however, results on low-dose exposure vary. A prospective cohort study on premenopausal women having a median mercury concentration of 1.1 µg/L, showed no association between mercury and kidney biomarkers such as BUN and creatinine (Pollack *et al*., 2015). In addition to urea and creatinine, Oda & El-Ashmawy, 2012 indicated a significant reduction in albumin on exposure to mercury through subcutaneous injection compared to the controls. As an observation, the reduction is attributed to a decline in protein synthesis (Oda & El-Ashmawy, 2012).

Cystatin C is a small molecular inhibitor of the cysteine protein family present in the plasma. It is produced by nucleated cells consistently. Due to its low molecular weight of approximately 13, 000 Daltons, it can easily permeate across the glomerulus membrane, hence, it molecular size is essential in its re-absorption at the renal tubules (Groesbeck *et al*., 2008). Its degradation occurs in the renal tubule, but not its secretion. These properties make it a suitable biomarker of kidney injury, reflecting alterations in kidney function and glomerular filtration. Just like urea, cystatin C concentration is dependent on variables like gender and age. In males, its concentration is higher than in females. Instead of increasing with age like creatinine, it shows a decrease in concentration (Groesbeck *et al.*, 2008). However, Knight *et al.* (2004) noted that factors such as gender, muscle mass, diet, infection, inflammation, and tumours may not influence cystatin concentration in the plasma. Only smoking, thyroid dysfunction,

immunosuppressant usage, and systemic inflammatory response could influence its concentration.

It is important to note that the initial cause of the kidney injury influences the period required for the cystatin C increase to be observed. This finding was based on human studies. In the case of a cardiopulmonary bypass, the increase was observed after 2 hours, while in cardiac surgery, 12 hours after the surgery, the increase was observed (Krawczeski *et al.*, 2011). Due to its specificity and sensitivity, it functions as an ideal index for renal function. However, due to a lack of standardized tests, its usage is limited.

Neutrophil gelatinase-associated lipocalin (NGAL) has been extensively examined as a biomarker due to its role in tubular regeneration and kidney development following an injury. Other than the kidney, it is widely distributed in other organs like the colon and liver (Cowland *et al*., 2003). It is a carrier protein, evident in its structure, that contains an eight-stranded beta-barrel with a pronounced cavity containing polar and charged amino acid residues. Activated neutrophils of the proximal tubule produce NGAL in the form of covalently linked gelatinase of neutrophils. It plays a role in the repair of renal tubules, lipid metabolism, and maturation of epithelial cells in the kidney tubules (Cowland *et al.*, 2003). Also, it has been linked to playing a role in host defence due to its expression in cells frequently in contact with microorganisms. Under normal physiological conditions, NGAL is produced in low quantities and is elevated in pathological conditions. In nephrotoxic conditions, it is significantly increased due to injury to the epithelial cells of the proximal tubules (Sanjeevani *et al.*, 2014).

In the blood, NGAL can be an early predictor of kidney injury due to its high specificity and sensitivity in cases of hematopoietic stem cell transplantation and heart surgical operations. As a predictor of acute kidney injury, it is more effective compared to

markers such as creatinine (Kim *et al.*, 2014). Its concentration in the blood is significantly elevated for individuals that develop acute kidney injury a week after a burn. In cases of severe burns, it functions as an early independent predictor of acute kidney injury (Sen *et al*., 2015). Since it is available in blood or plasma, it has its merits and demerits. Though plasma is readily available, drawing blood makes the process invasive. Also, as mentioned earlier, its concentration may depend on systemic inflammation, malignancy, and bacterial infections (Iguchi *et al*., 2015). Hence, its application is favourable in pathological instances with fewer complications.

B2-microglobulin (B2-M) also stands out as a molecule that is freely filtered by the glomerulus and its reabsorption occurs in the proximal tubule where it is metabolized. It is a small molecule (11,800 Daltons) produced by nucleated cells, with high quantities in immune cells such as monocytes. Concentrations of B2-M are increased in kidney disease, and a similar trend is observed in ageing, autoimmune conditions, and malignancies (Shinkai *et al*., 2008). As a glomerular filtration marker, in instances of reduced glomerular filtration its serum concentration increases as observed before serum creatinine level increases. In terminal conditions in elderly patients, it functions better as a biomarker compared to cystatin C even after adjusting for other risk factors (Lopez-Giacoman & Madero, 2015).

A protein also filtered by the glomerulus and reabsorbed in the proximal tubule is the beta-trace protein (BTP), which fits the criteria for determining glomerular filtration. It is a lipocalin, a glycoprotein of low molecular weight (23,000 to 29,000 Daltons). Its concentration in the serum is increased in renal disease states. As a measure of decreased glomerular filtration, it can function better than serum creatinine (Woitas *et al*., 2001). Compared to cystatin C, however, it is an inferior indicator of renal function (Priem *et al*., 2001).

The above-mentioned markers of kidney function and injury can be effective for assessment purposes. They are indicators of pathological processes, biological processes, or pharmacological responses in therapies.

2.3. Histological changes associated with mercury-induced kidney injury

Traditionally, histopathology functions as a means of identifying morphological changes relating to a diagnosis, response to therapy evaluation, and basic research. Studies have linked renal morphological alterations with heavy metal intoxication (ATSDR, 1999). In toxicological investigations, including heavy metal toxicities, histological examinations have functioned as reliable biomarkers (Gökc *et al*., 2016). Usually, the effect of exposure to toxic metals such as mercury is necrosis and apoptosis in cells of different types and autophagy resulting in cell death. The intracellular targets include the endoplasmic reticulum, cytoskeleton, mitochondria, and acidic compartments (Vergilio *et al*., 2015). The alterations are evident at the tissue level in experimental animals (Gökc *et al.*, 2016). The kidney is a key organ essential for the maintenance of homeostatic conditions. Due to its sensitivity, it is vulnerable to drugs and environmental chemicals, causing renal injuries, indicating that nephrotoxic effects of inorganic Hg forms are always rapidly evoked (Gökc *et al*., 2016). In mammals, bio-accumulation is prominent in the kidney, but there is still more to be learned about the handling of mercury by the kidney.

Mercury in the form of mercuric ion binds strongly to thiol-containing groups and increases reactive oxygen species, causing oxidative stress and leading to tissue damage. Specifically, it is associated with the reduction of glutathione, the formation of superoxide radicals, and hydrogen peroxide (Agarwal *et al*., 2010a). The most vulnerable segment of the nephron to the toxic effects of inorganic Hg is the pars recta (the straight descending section of the proximal tubule). The pars recta exhibit necrosis

and epithelial injury in experimental animals. Sometimes the distal segment is also affected (Zalups, 1991; Zalup, 2000). The organelle principally affected in the proximal tubular system is the mitochondria, evident by the swelling of mitochondria, defective oxidative phosphorylation process (Weinberg *et al*., 1982), and reduced ATP concentration. The presence of mercury affects the structural integrity of the mitochondrial inner membrane, which is often followed by a reduced concentration of reduced glutathione and the promotion of hydrogen peroxide generation by the electron transport chain, further leading to lipid peroxidation (Lund *et al*., 1993). Depending on the extent of the deterioration of kidney function, necrotic damage and cellular injury are observable in the whole of the pars recta. Specifically, starting from the Bowman's capsule to the outer medulla.

The changes are specific to the section at the cortex and outer medulla junction. Initial pathological changes in the epithelial cells along the proximal tubule include an increase in the size of cells, vacuolization, and nuclear swelling. In the early stages, the proximal tubular epithelium's degenerative changes are normally accompanied by minor toxic damages and regeneration processes. Tubular dilation, thickening of the tubular basement membrane, and desquamation of the epithelial cells indicate the progression of the injuries that are then followed by inflammation, necrosis, and atrophy of the tubules and fibrosis (ATSDR, 1999; Nordberg *et al*., 2005; Zalups, 2000).

Inorganic mercury is majorly nephrotoxic, and as little as 0.5 grams can bring about necrosis in the renal tubules of humans. In extreme situations, the result is death. Along the segments of the proximal tubule, degenerative alterations are observable as early as one hour following exposure to 100 mg $HgCl_2$ per kilogram dose, which is considered a very high dose (Bridges & Zalups, 2017). At the lowest concentration (2.5 µmol of

$HgCl_2$ per kg or 0.5 mg of $HgCl_2$ per kg body weight) in experimental animals, the structural damage is limited to the pars recta (Nicholson *et al.*, 1985). For lower doses (1-5 mg of $HgCl_2$ per kg body weight), observable light microscopy pathological changes are not evident till 6-8 hours after exposure (Bridges & Zalups, 2017). However, through electron microscopy, the proximal straight tubule shows alterations at the cellular level three hours after a subcutaneous injection of 4 mg $HgCl_2$ per kg dose (Zalup, 2000). These alterations include a decrease in the mitochondria matrix granule with swelling of the matrix, rough endoplasmic reticulum enlargement, cisternae dilation of the smooth sarcoplasmic reticulum, and ribosomal dispersion. Notably, at the end of the twelve hours after initial exposure to mercuric ions, cellular necrosis is observable through both electron and light microscopy (Zalup, 2000). At sub-acute lower doses of mercuric chloride (0.02 mg per kg body weight), glomerular lobulation and tubular dilation are evident in the kidneys of experimental rats after 28 days (Gökc *et al.*, 2016).

The involvement of the proximal tubules and partly the distal nephron portions is evident in severe cases of nephropathy. Changes in the distal segments of the nephron show effects secondary to the severe injury observed on the pars recta (Zalup, 2000). An increase in the dosage leads to more pronounced degenerative and necrotic alterations. Such observations are made in the most proximal regions of the proximal tubules (Nicholson *et al.*, 1985). Necrotic cells were observable at the end regions of the pars convolute. In the medullary collecting duct and distal tubules, limited tubular cell casts were observable. At higher concentrations, the collecting and distal ducts experience obstruction with exfoliated necrotic cellular debris due to necrosis in the more proximal sections (Nicholson *et al.*, 1985). The cells of the more proximal regions experienced nuclear pyknosis (shrinking of the nucleus) with the loss of micro-villi.

Instances of epithelial cell loss left only the basic tubular structure based on the basal lamina (Nicholson *et al.*, 1985). In animal studies, for a very high dose of $HgCl_2$ (0.1 mg of $HgCl^2$/g body weight), initial degenerative changes in the proximal tubules are observed as early as 1 hour after exposure.

Exposure to mercuric ions can alter the appearance and weight of the kidney in animal models. Rats' kidneys exposed to mercuric ions (7.5 and 10 µmol/kg or 1.5 and 2 mg of $HgCl^2$/kg body weight) tend to be paler and slightly swollen. Observation of the transverse section indicated a necrotic tissue band at the corticomedullary junction. The pale band marks the pars recta region of the proximal tubule (Nicholson *et al.*, 1985). Based on the findings by Nicholson *et al*. (1985), the mean weight of paired kidneys exposed to mercury is increased compared to the control.

In a study focusing on mercury vapour inhalation in specially designed glass cages for forty-five days, histopathological findings showed nucleus pyknosis in tubular and glomerular cells, vacuolar changes, and tubular necrosis in rats. Glomerular sclerosis and degeneration were also evident. In comparison to the control groups, there was a dilation in the Bowman's space. Dead cells and structures made the collecting tubule and cytoplasm borders indistinguishable. These observations were based on light microscopy (Akgül *et al.*, 2016). Electron microscopy findings indicated structural changes in the mesangial cell (with a hyper-chromatic nucleus), podocytes, and distal tubule cells. The tubule cells showed a progressive degeneration of the mitochondria. The basement membrane and glomerular cells appeared damaged, meaning that the podocytes exhibited hyper-chromatic nuclei, irregular membranes (thickened), and vacuolar degeneration (Akgül *et al*., 2016).

Long-term mercury exposure can bring about membranous nephropathy which is a condition affecting the glomerular basement membrane through the immune deposits

accumulation in the cortex. Observation using a light microscope shows a thickened glomerular basement membrane and slightly proliferative mesangial cells (Li *et al.*, 2010). Also, kidney injury due to mercury exposure causes dose-dependent tubular dysfunction and idiosyncratic nephrotic syndrome. On a case report, a biopsy indicated 22 per cent sclerotic glomeruli on a global scale using light microscopy. 27 per cent of glomerular increased thickness of the capillary wall mesangial cell and matrix expansion. Hyalinosis and sclerosis with adhesion to the Bowman capsule were evident on a segment of a single glomerulus (Miller *et al.*, 2013).

In non-fatal cases with exposure to nephrotoxic doses, there is a complete regeneration of the proximal tubule epithelium, that is, relining of the proximal tubular epithelium in the first two weeks following the induction of tubular degeneration. In an experimental study, however, the regeneration - relining of the epithelium - occurred as early as four days following an administration of a 1.5 mg $HgCl_2$ per kg dose intravenously (Zalup, 2000).

2.4 Management of mercury toxicity

2.4.1 Prehospital care

According to ATSDR, prehospital measures are essential such as the immediate removal of the affected individual from the contaminated environment (ATSDR, 1999). There are immediate considerations and decontamination options that are worth considering. In the primary management of acute toxicity due to exposure to Hg°, observing vital organs is important. The individual can be placed under intubation and mechanical ventilation for the supplementation of oxygen. In this initial phase of management, bronchoalveolar lavage (washing the airways with a saline solution) is discouraged to limit further spread of Hg and increased absorption in the lungs. Then the extent of the spread is determined through a chest X-ray procedure. Usually, the

rate of absorption is inversely proportional to the size of the Hg droplets (Bates, 2003). In instances of acute exposure through ingestion intravenous therapy through fluid replacement limits shock as a result of the mercury induced injuries. In order to determine the extent of the visible toxic injuries, an endoscopic examination is prescribed, which can diagnose oedema and / or an obstructions of the airways in the case of acute inhalation. In the presence of an obstruction, tracheostomy, intubation, and/or intravenous fluid therapy will be of essence (Rafati-rahimzadeh *et al.,* 2014). Direct contact with skin requires thorough washing with soap and water.

Decontamination is mainly applicable in ingestion of inorganic mercury to limit absorption in the gut. Though, the major problem is the corrosive nature of inorganic mercury compounds, it does not make the decontamination process inessential. Still the removal of the residual mercury is important using a polyethylene glycol solution (Rafati-rahimzadeh *et al.,* 2014). Similarly activated charcoal can be used, however, its efficiency is not certain due to its limited ability to bind heavy metal. After decontamination, radiographies of the abdominal region are essential in follow-ups (Kleinschmidt *et al.,* 2011).

2.4.2 Chelation therapy

Chelation therapy is sometimes a consideration depending on the dose. Various chelating agents are used in cases of acute inorganic Hg exposure, especially in adults. They include: penicillamine, *N*-acetylcysteine, dimercaprol, dimercaptosuccinic acid, and dimerptopropane sulfonic acid. Among children dimercaptosuccinic acid is allowed for usage as the preferred chelator by Food and Drug Administration (FDA) (Rana *et al.,* 2018).

Penicillamine is a hydrophilic penicillin derivative and often its D-isomer is preferred to the L-isomer due to its less toxic nature. The D-penicillamine has found application

in the treatment of Wilson's disease, a genetic disease in which copper accumulates in vital organs, and in management of other heavy metals (Yusof *et al.*, 2000). It functions by promoting excretion of heavy metals like mercury and lead. Among the different forms of mercury, D-penicillamine is only applied in the management of elemental mercury and inorganic mercuric salts. Its usage presents side effects such as kidney injury and hypersensitivity. Analogs have been developed for improved effectiveness, for example, *N*-acetyl penicillamine that is a better mercury chelator. D-penicillamine is less preferred compared to dimercaptosuccinic acid or succimer since it has less adverse effects and a stronger metal binding capacity (Rafati-rahimzadeh *et al.,* 2014). *N*-Acetylcysteine (NAC) can be used as a chelator in managing mercury toxicity. It is also presented as a free radical scavenger and an antioxidant, with the ability to improve glutathione content (Rossignol, 2019). Its most desirable quality is its ability to reduce heavy metals concentration in tissues without promoting excretion of essential minerals or metals making it a safer chelator. There are experimental studies that show the potential of NAC as a mercury chelator. The presence of NAC in rats exposed to mercuric chloride decreased hepatic and renal mercury content as illustrated by Girardi & Elias (1991). In a study focusing on exposure to mercury vapour, treatment of the rats with NAC prolonged their lives in the presence of mercury and the blood mercury levels were reduced (mercury levels were approximately twice higher in the non-treated group compared to the NAC-treated group at the end of the experiment) (Livardjani *et al.,* 1991). Coadministration of NAC and zinc in rats exposed to mercury limited retention of mercury in the blood and liver (Oliveira *et al*., 2015). Though these studies show effectiveness there is a report of no improvement while using NAC as a chelator. In a study exploring the effects of NAC and dimercaptopropane sulfonic acid (DMSA) on the liver, kidney, and blood of mercury exposed rats, both the use of both chelators

presented kidney tissue damage. The investigator justified this observation to the formation of toxic complexes (Brandão *et al.*, 2006). In humans, the use of NAC in mercury exposure has presented improvements. In a case of a woman who ingested elemental mercury, treatment with NAC improved the symptoms, which were, abdominal discomfort, fever, and diarrhoea (Sarikaya *et al.*, 2010).

One of the oldest treatment for heavy metal toxicity is the British anti-lewisite (BAL) or dimercaprol. Its application included the treatment of Wilson's disease, though it's commonly used in the management of heavy metal toxicities (Vilensky & Redman, 2003). The first case of its application was on a patient acutely exposed to mercuric chloride through ingestion. In a case series involving patients exposed to mercuric chloride, in the absence of BAL, twenty-seven out of eighty-six patients died after consuming mercuric chloride equal to or more than one gram and treated using conventional supportive methods (Kosnett, 2013). Treatment with BAL prevented death among forty-one patients who have ingested mercuric chloride equal to or more than one gram. This case mortality rate equates to 31.4 percent. The patients were presented within four hours. As a chelator it is less effective and has a tendency of increasing the concentration mercury in the brain and promote mercury's effects on the central nervous system on exposure to organic mercury (Rafati-rahimzadeh *et al.*, 2014). The adverse effects include headache, vomiting, hypertension, convulsions, and increased heart rate (Brent *et al.*, 2017).

Dimercaptosuccinic acid (DMSA) is an enzyme inhibitor and hydrophilic in nature. The inhibition is against enzymes containing the sulfhydryl functional group and the outcome is prevention of mercury induce effects. It is easily metabolized in humans and the main route of excretion is the through urine with a half-life of about three point two hours. Only a negligible amount is excreted by respiration and via bile (Rooney,

2007). Due to these properties, DMSA administration is a preferred treatment to mercury poisoning (Kleinschmidt *et al.*, 2011). The commencement of its administration should start in children having a urine Hg concentration equal to or higher than 50 μg/kg of urine creatinine. This recommendation is inclusive of asymptomatic cases (Forman *et al.*, 2000). The administration is either intravenous or oral for a span of 19 days and in some cases the treatment is repeated after a two-week interval. Though the compound is minimally toxic in its chelating activity still there are some side effects that exist. They include skin rashes and gut disorder. A distinct side effect is decreased neutrophil count which possess the need for complete blood count, renal, and liver function tests before treatment and in the course of treatment (Rooney, 2007).

A derivative of dimercaprol used in the management of mercury poisoning is dimercaptopropane sulfonic acid. In some cases, it functions as a replacement for DMSA (Kleinschmidt *et al.*, 2011) since it is safer than DMSA. Hence it is widely used and exists as an over-the-counter drug (Bernhoft, 2012). Compared to DMSA its metabolism is faster, wherein the initial thirty minutes eighty per cent of DMPS is metabolized. Though in some cases ninety per cent of the absorbed DMPS is metabolized. The uptake of DMPS from the gut ranges from 39 - 60 % (Bernhoft, 2012). Its metabolism involves its conversion into disulfide forms. The disulfide forms of DMPS have an excretory half-life of approximately ten hours, while that of the unaltered form is approximately four point four hours. However, these observations are varied in different cases (Bernhoft, 2012). The major route of excretion is via urine, which accounts for eighty-four per cent of the total DMPS.

Upon entry into the kidney tubular cells, it binds Hg reducing the mercury burden in the kidney (Vamnes *et al.*, 2003). The process involves DMPS penetration into the

renal cells, removal of the Hg present in the renal cells or tissues, and the excretion of Hg into the urine. The excreted compound is a complex of inorganic mercury and DMPS (a chelating agent). According to scientific pieces of evidence, DMPS is incapable of removing mercuric mercury present in the brain after exposure, unlike other organs (Rooney, 2007). Similar to DMSA its administration is either oral or intravenous. Often, oral therapy precedes intravenous administration and the treatment duration relies on the mercury body burden. Though rare, the side effects range from decreased white blood cell count, and nausea, to rashes (Brent *et al.,* 2017).

In addition to the above-mentioned chelating agents, there exist DMSA analogues that find application in managing Hg poisoning. DMSA are among these compounds which can either be mono or di-esters. This property improves their removal from the tissue compared to DMSA which cannot pass through the cell membrane. However, DMSA can tightly bind Hg through its sulfhydryl group removing from bile and kidney and it has both hydrophobic and lipophilic properties (Rafati-rahimzadeh *et al.*, 2014).

Among the esters of DMSA, mono isoamyl ester is better in reducing the Hg burden in the body through chelation. Its advantage is primarily through its ability to access intracellular spaces allowing for extensive cellular distribution. Hence, it removes Hg from both extracellular and intracellular spaces (Flora *et al.*, 2012). Additionally, the DMSA mono isoamyl ester lowers oxidative stress through two mechanisms. The first is through the reduction of body burden as mentioned above since heavy metals bind strongly to the sulfhydryl group and the second is by reducing reactive oxygen species through the sulfhydryl functional groups (Flora *et al.,* 2012). Its lipophilic characteristic and small molecular size allow for better permeability through membranes during the removal of heavy metals hence better effectiveness in therapy. Unlike DMSA, its administration is oral and intraperitoneal. However, oral administration tends to be

more effective compared to intraperitoneal administration based on studies examining the kidneys and liver of experimental animals (Flora & Pachauri, 2010). Monocyclohexyl DMSA and monomethyl DMSA are the other derivatives of DMSA. They easily cross cell membranes due to their lipophilic nature and reduce mercury's body burden through chelation. Unlike the other analogues, its administration is only oral. Though they are considered in chelation therapy additional experimental studies are required to ascertain their effectiveness (Flora, 2009).

In the management of acute intoxication with inorganic mercuric salts, animal experiments offer a good perspective on the effectiveness of chelators (Kosnett, 2013). In an experimental study ninety per cent of the rats in the positive control group were exposed to a very high mercuric chloride dose, that is, 109 mg per kg through intraperitoneal injection. The introduction of a single BAL, DMSA, and DMPS within fifteen minutes after exposure limited the deaths to fifty, forty, and zero per cent respectively (Nielsen & Andersen, 1991). In an experimental model focusing on acute kidney injury on exposure to a lower mercury dose of 1.4 mg per kg body weight administered intravenously, immediate administration of DMPS of concentrations 54 mg per kilogram body weight prevented kidney failure characterized by low urine output. A delay in the administration of DMPS by 24 hours makes the protective effect ineffective (Wannag & Aaseth, 1980). Hence, there is time dependence on the effectiveness of chelators.

2.4.3 Combination chelation therapy

Due to the limitations of the administration of a single remedy, combination therapy can be helpful. It is increasingly becoming a consideration in the management of heavy metal toxicity. Administering mono isoamyl DMSA in combination with DMSA shows increased effectiveness compared to mono isoamyl administration alone. The co-

administration limits oxidative stress through the management of lipid peroxidation and reduced catalase enzymatic activity. With the increased effectiveness there is a decrease in the dose administered that is associated with the improved patient recovery and a decrease in the occurrence of side effects (Bhadauria & Flora, 2007).

2.4.4 Selection of a chelating agent

The choice of a chelator is dependent on the mercury form. Often the thiol chelators (DMSA and DMPS) are preferred in cases of elemental mercury poisoning. They increase urinary mercury output, decrease mercury content in blood, and the kidney (Bradberry *et al.*, 2009). In reducing brain mercury levels following an exposure to mercury vapour, both thiol chelators and acetylcysteine show ineffectiveness (Spiller *et al.,* 2021), partly explaining the variations observed in clinical outcomes after an exposure to elemental mercury vapour However, in acute asymptomatic cases the patient remains asymptomatic even with increased urinary excretion and decreased blood mercury concentration (Forman *et al.*, 2000). Additionally, in acute exposure instances the thiol chelators are ineffective in the management of pulmonary injuries (Beck *et al.,* 2004). A similar pattern is observed in both mercury vapor intoxication and elemental mercury injection (Spiller *et al.,* 2021). Though acetylcysteine appears not to decrease mercury concentration in tissues following a mercury vapour intoxication it limits organ damage according to experimental animal studies. The proposed justification for this observation is increased glutathione content within cells (Livardjani *et al.*, 1991).

The choice of a chelator is also applicable in mercuric salts exposure. Experimentally, DMPS administration after mercuric chloride injection reduced mercury deposits in all organs. On the other hand, DMSA only reduced mercury quantity in the kidney and blood. The treatment had to start within 24 hour of exposure to mercury (Bridges *et al.,*

2011). A delay in treatment meant a reduction in the effectiveness of the chelator (Aposhian *et al.,* 1996), an observation pointing to the limitation in tissue penetration of the thiol chelators. Also mercury redistribution occurs following a prolonged administration of the thiol chelators as aforementioned, leading to increased mercury content in neuronal tissues (Spiller *et al.,* 2021). Acetylcysteine shows varying results after mercuric chloride administration. In one case it did not influence the mercury content in organs (Ballatori *et al.,* 1998) and in another the reduction in mercury content was only in the liver and kidney, accompanied by improved function of these organs (Joshi *et al.,* 2014). In humans, the thiol chelators decrease blood mercury level and increase urinary mercury excretion significantly after inorganic mercury salt ingestion and exposure through skin (Spiller *et al.,* 2021). Based on the properties of the chelators, the thiol chelators can be used in the first three to four days following elemental mercury and inorganic mercury salts exposure, mainly to reduce renal mercury content. Switching to acetylcysteine is appropriate after the three to four days for limiting organ injury and continuation of the chelation process.

2.4.5 Limitations of chelation therapy

Mercury chelation as an approach to managing mercury toxicity has limitations and concerns. Among the chelators, DMSA and DMPS are relatively tolerable on administration. In addition to the aforementioned information on contraindications, including allergic reactions, mainly, skin rashes account for one to ten per cent of subjects in various studies that have been reported. Mild gut complaints instances are observed in some patients. Though mild increases in hepatic transaminase and a decline in leukocytes are observed as isolated cases they are reversible (Kosnett, 2013). Due to their lack of specificity the chelators, DMPS and DMSA, have been associated with increased copper and zinc excretion from the body (Smith & Strupp, 2013). Since

chelation effectiveness in cases of acute inorganic mercury exposure reduces with the increase in the time difference between exposure and the commencement of treatment, there is the need for an initial dose administration promptly before laboratory confirmation is obtained when there is a high diagnostic suspicion irrespective of the contraindications (Kosnett, 2013).

In chelation therapy, the use of chelators aims at mobilizing heavy metals, however, this mobilization does not equate excretion of the heavy metals. Thiol chelators, that is, DMSA and DMPS administration following chronic mercury exposure reduces blood mercury concentration and increase mercury excretion in urine but do not equate to an improved clinical outcome (Gul Oz *et al.,* 2012; Spiller *et al.,* 2017). The probable justification for the observation is, the alterations in urine and blood mercury concentrations do not address changes such as inhibition of selenoproteins, reduction in cellular selenium content, and mitochondrial injuries associated with these changes (Spiller, 2018). Some chelators play a role in the redistribution of some heavy metals, mercury included, in the body. Even with increased heavy metal excretion, the heavy metal redistribution in tissues can still have a negative impact (Kosnett, 2013). Considering the case of BAL as earlier mentioned is a representation of this phenomenon (Rafati-rahimzadeh *et al.,* 2014). Thiol chelators such as DMPS and DMSA are incapable of removing mercury directly from affected organs like the liver and brain. This limitation is associated with their inability to penetrate the tissue of these organs.

In clinical setups, the use of chelation therapies in mercury intoxication promote mercury excretion through urine, however, the patient reports on the outcome show conflicting observations. Some showed improved kidney, neurological, or cardiovascular function (Bradberry *et al.,* 2009; Mercer *et al.,* 2012), limitation of

mercury toxic effects (Vallant *et al.,* 2008), no changes or improvements (Brannan *et al.,* 2012; Carter *et al.,* 2017), and others showed increased deterioration (Gul Oz *et al.,* 2012; Spiller *et al.,* 2017). This inconsistency observed during chelation presents the reconsideration of relying on blood and urine levels to assess the effectiveness of chelation therapy. Mainly because, while there are patients whose health status have improved significantly after chelation therapy, including patients not showing increases mercury presence in urine and blood, also, there are cases of reports showing no improvements and deterioration (Spiller *et al.,* 2021).

Other than the comparison of blood and urine mercury concentration, there are factors that can affect the clinical outcome. The different forms of mercury, which are, inorganic mercury salts, elemental, and organic mercury, show difference in potency, target organ, clinical manifestation, and toxicokinetics. Considering the duration of exposure that allows for mercury's distribution to target organs is important (Spiller *et al.,* 2021).

The application of chelation therapy after an acute exposure to high concentrations of elemental mercury vapor still presents uncertainties in the field of toxicology. Through animal studies DMPS and DMSA show ineffectiveness in reducing mercury deposits in the brain after mercury vapor inhalation (Kosnett, 2013). In an elaborate study focusing on DMPS and DMSA chelation in experimental rats after an inhalation of elemental mercury vapor, most mercury content reduction were observed in the kidney, though there was not a reduction in brain's mercury content (Buchet & Lauwerys, 1989). This observation points to the limited application of chelation in management of the neurological effects associated with chronic or acute exposure to high doses of elemental mercury. It is safe to say that DMPS and DMSA effectively reduce the mercury deposits in the kidney based on animal studies. In human they increase

mercury excretion through the urine (Gonzalez-Ramirez *et al.,* 1998), though it is unclear if they reverse kidney injury brought a chronic exposure to high doses.

2.4.6 Other treatment options

In extreme conditions of exposure where the patient's state of health is critical and there are no preferred therapy options plasma exchange is considered. These are emergency conditions where the body burden is high, making it potentially useful in managing heavy metal toxicity. The process of plasma exchange commences 24-36 hours after diagnosis (Russi & Marson, 2011). In cases of renal failure, hemodialysis is a treatment option especially for hydrophilic and dialyzable compounds (Nenov *et al.,* 2003). If heavy metals are strongly bound to plasma proteins hemodialysis is ineffective. The recommended therapy will be plasmaphoresis that is capable of removing heavy metals strongly bound to plasma proteins. Combining these therapy procedures with chelation is recommended since with chelation therapy alone the mercury's elimination half-life is between thirty to a hundred days while in combination with hemodialysis the elimination half-life drops to two to eight days (Dargan *et al.,* 2003).

2.5 Use of phytochemicals and plant extracts as a strategy in reducing the Hg^{2+} induced nephrotoxicity

For years, multiple approaches have been considered to determine the possible effectiveness of plants against Hg toxicity to the kidney. Various plants or phytochemicals displayed nephroprotection by limiting Hg accumulation and oxidative stress and increasing thiol content (Rana *et al*., 2018). Since interference with the endogenous thiol status resulting in oxidative stress is a mechanism of inorganic mercury toxicity, antioxidants could be used to manage the toxicity. Antioxidants have been known to limit the effects of free radicals on the body. Even antioxidants derived from plant sources are essential in maintaining good health.

2.5.1 Use of phytochemicals

The ameliorative and protective potential of vitamin E has been observed in $HgCl_2$-administered rats, whereby post-treatment was more effective than pre-treatment. The investigation focused on renal markers of oxidative stress and the activity of the antioxidant enzymes; serum biomarkers of real damage; metallothionein-II (MT-II) and metallothionein-I (MT-I) mRNA expression with mercury accumulation and histological alterations in the kidney and liver (Agarwal *et al.*, 2010b). Other than mercury, vitamin E has been effective in other metal-mediated tissue injuries such as cadmium and copper. Vitamin E's effectiveness against lipid peroxidation has been demonstrated by Valko *et al*. (2005).

The inhibition process involves scavenging for lipid peroxyl radicals and reacting with them to form tocopheroxyl radicals. This product can either be reduced to alpha-tocopherol by an antioxidant or further oxidized to alpha-tocopheryl quinone, an anticoagulant or antioxidant which forms hydroquinone after reduction (Arita *et al.*, 1998). The protection is also seen in the reversal of affected glutathione concentration. In the kidney, vitamin E post-treatment can minimize renal mercury retention with no protection against the generation of oxidative stress. Complementing the findings on oxidative stress is the increased expression of metallothionein-II (MT-II) and metallothionein-I (MT-I) mRNA, signifying increased binding of mercuric ions by metallothioneins (Agarwal *et al*., 2010b).

Curcumin, a hydrophobic polyphenol found in *Curcuma longa* (turmeric plant), has been shown to be effective against the harmful effects of heavy metals, mercury included. The polyphenol prevents tissue damage, depletion of glutathione, lipid peroxidation, and maintains the antioxidant enzyme system. These protective effects are accounted for by its antioxidant and chelating properties (Agarwal *et al*., 2010a;

García-Niño & Pedraza-Chaverrí, 2014). Superoxide dismutase is often elevated due to increased hydrogen peroxide generation by mercuric chloride. Interestingly, curcumin depletes or inhibits superoxide dismutase. The inhibition can either be linked to the covalent binding of mercuric ions to cysteine residues in the enzyme (Shimojo *et al.*, 2002) or the unavailability of zinc and copper, which are essential in the detoxification of mercury due to their interaction with metallothioneins (Brzóska *et al*., 2002).

There is also the possibility of superoxide dismutase inhibition being the product of oxidative conditions that negatively influence the structure of the enzyme. A similar pattern is noted in the activity of glutathione peroxidase where its activity is lower than the control group, due to the decreased superoxide dismutase catalysis. Also, catalase activity was reduced to normal levels (between 100-120 micromoles CAT/min/mg protein) and lipid peroxide levels were restored (between 2-4 nanomoles MDA/mg protein). Based on the histological findings, there was no tissue protection. It was hypothesized that since the repair of tissue damage is time-dependent, the limited time did not allow for the reversal of the tissue changes (Agarwal *et al*., 2010a).

Since Arabic gum has been beneficial in the management of renal failure and nephrotoxicity it has been studied to examine its nephroprotection against $HgCl_2$-induced nephroprotection. In the traditional Arabic medicine, it has found application in the management of chronic renal failure by limiting the need and frequency of hemodialysis (Gado & Aldahmash, 2013) and experimentally it offered protection against gentamicin-induced nephrotoxicity by limiting lipid peroxidation (Al-Majed *et al.*, 2002). The nephroprotection was against a $HgCl_2$ dose of 5 mg/kg body weight that significantly increased the concentrations of blood urea, serum creatinine, total nitrate/ nitrite and thiobarbituric acid reactive substances (TBARS). Additionally, antioxidant

system activity was reduced as indicated by the reduction in the reduced glutathione, catalase, and glutathione peroxidase enzymes (Gado & Aldahmash, 2013).

Increased TBARS points to increased lipid peroxidation since $HgCl_2$ plays a role in increasing reactive oxygen species (ROS) production that enhances lipid peroxidation followed by tissue damage. Also, reduced glutathione forms complexes with Hg and reduced ROS. accounting for depletion in the presence of Hg in the body (Gado & Aldahmash, 2013). Reduction in the activity of glutathione peroxidase, catalase, and superoxide dismutase accompanies a decrease in reduced glutathione. The restoration of TBARS and glutathione shows antioxidant properties, meaning the Arabic gum components can scavenge free radicals. The scavenging limits lipid peroxidation and the resulting tissue injuries (Gado & Aldahmash, 2013).

In a single study, pretreatment with tea polyphenols and Schisandrin B was explored to determine their nephroprotective effect against a varying concentration of $HgCl_2$. $HgCl_2$ increased the activities of *N*-acetyl glucosaminidase (NAG), lactate dehydrogenase (LDH), and alanine phosphatase (ALP). Other observations included a reduction in the activity of the endogenous antioxidants, increased generation of ROS accompanied by lipid peroxidation and apoptosis, and histological degeneration. Pretreatment with schisandrin B and tea polyphenols reduced the oxidative stress, activities of ALP, LDH, and NAG, and restoration of the histological changes (Liu *et al.*, 2011). This experiment was based on the premises that $HgCl_2$ injection induces dose-dependent necrosis and apoptosis (Kanda *et al*., 2008), acute renal failure where proximal tubules are mainly affected (Zalup, 2000), and has a strong link with free radical generation and lipid peroxides destroying the integrity of cell membranes. Additionally, tea polyphenols tend to limit oxidative stress associated with diseases like cardiovascular disorders and cancer. Primarily, these phenols react with reactive

oxygen species that influence various biochemical reactions such as apoptosis. It is important to note that among the polyphenols in tea epigallocatechin-3-gallate is the most effective due to its ability to interact with many free radicals and limit their generation (Yang *et al*., 2009). Similarly, schisandrin B can remove free radicals and limit lipid peroxidation. These properties have made consideration of protective agents in the management of exogenous and endogenous oxidants that cause tissue damage (Chiu *et al.*, 2006).

N-acetyl cysteine, amino acid cysteine linked to an acetyl group, protects tissues in the kidney, liver, and lungs against mercuric chloride-induced oxidative damage by reversing the responses due to oxidative stress. The results were achieved by determining malondialdehyde and glutathione levels and the activity of myeloperoxidase (Özer & Ayanogˇlu-Dülger, 2003; Nava *et al.*, 2000). Its administration limits the oxidation of proteins and lipids and reduces tissue glutathione levels. In addition, *N*-acetyl cysteine often donates a sulfhydryl group in the synthesis of glutathione and inhibits the generation of oxygen radicals in the extracellular spaces (Dobrzynska *et al.*, 2000). It can also react with free radicals directly. Though in some cases, the reduced concentration of the reduced glutathione is incapable of causing lipid peroxidation, it could enhance other factors that promote the peroxidative process (Özer & Ayanogˇlu-Dülger, 2003).

Because of the similarities in the metabolism of *N*-acetyl cysteine and reduced glutathione, part of the beneficial effects of *N*-acetyl cysteine can be linked to limiting lipid peroxidation. Its beneficial effects are connected to its free radical scavenging activity. Therefore, its administration can offer protection to the kidney, limiting protein and lipid oxidation while promoting clearance of mercuric chloride from the kidney (Girardi & Elias, 1991).

Another cytoprotective antioxidant against $HgCl_2$-induced nephrotoxicity is L-carnitine. It brought about a reversal of the mercury-induced rise in serum creatinine and urea. These biological data were supported by histopathological examinations where the intensity of the damage was minimized (Gado *et al*., 2014).

In an in vitro study, quercetin, a plant flavonoid, was shown to reverse the redox status alterations due to the cytotoxic properties of mercuric chloride and methyl mercury (Rafael *et al*., 2011).

2.6 Medicinal effects of *Cleome gynandra*

Cleome gynandra Linn is among the medicinal plants of significance in pharmacological and traditional settings. The pharmacological applications in experimental studies include its use as an anti-diabetic, immuno-modulator, anti-cancer, anti-oxidant, anti-inflammatory (Adhikari & Paul, 2018), and anti-microbial agent (Van Vuuren & Holl, 2017).

2.6.1 Anti-diabetic property

Ravichandra *et al*. (2014) showed the anti-diabetic property of *Cleome gynandra* in alloxan-induced diabetic rats. Oral administration of *C.gynandra* extract significantly reduced the glucose profile. It ascertains its traditional usage. *C. gynandra* extract encouraged recovery compared to metformin, an anti-diabetic drug. The elevation of glucose concentration was limited. The increased blood glucose was due to the reduced insulin secretion as a result of the destruction of pancreatic beta cells by radicals due to alloxan's presence.

A herbal formulation having bio-available trace elements capable of managing glucose resistance can potentially increase its ability to improve diabetic conditions. Herbs with anti-diabetic properties can promote the activity of the pancreatic beta cells and also their regeneration (Ravichandra *et al.*, 2014). Essential nutrients such as magnesium,

sodium, zinc, and copper function as fundamental supplements essential for diabetes management (Das & Ahmed, 2017). The anti-diabetic property is tied to the plant's nutritive, immuno-modulatory, and antioxidant properties. Based on these properties, natural products can provide a synergistic remedy for life-threatening situations like diabetic nephropathy due to chronic diabetes (Adhikari & Paul, 2018).

The polyphenolic compounds are probably active phytochemicals vital for glucose oxidation-enhancing pathways. The constituent phytochemicals lack a significant influence on the plasma glucose level; however, they all together can reduce plasma malondialdehyde and other redox markers, with a considerable proteinuria moderation. This can both be referenced to polyphenolic compounds and flavones from aqueous and alcoholic extracts (Adhikari & Paul, 2018).

2.6.2 Immuno-modulatory property

Cleome gynandra alcoholic and aqueous extracts can limit significant increase in serum immunoglobulin G and M. However, the alcoholic extract is more effective, even in lower quantities. Generally, there is an immunosuppressive activity of both extracts, as indicative of the inhibitory effect shown by T cell-dependent antigen. *Cleome gynandra* ethanolic extract shows better efficiency by inhibiting about 92.74% of cell-induced hypersensitivity in an investigation of cell-mediated immunity in albino rats (Adhikari & Paul, 2018). Immune response regulation, either by suppressing or stimulating the immune system, can help keep diseases at bay. Therefore, agents promoting the body's defence against a defective immune system can support the existing techniques or approaches, especially during chemotherapy (Kori *et al*., 2009). Interestingly, in the experimental setups, suppression and modulation of the immune system after immune hyper-reactivity and drug-induced immunosuppression, respectively, are both considered to be regulators of the immune system (Labadie *et al*.,

1989). Although there is extensive information on phytochemicals capable of stimulating the immune system, information on immunosuppressive phytochemicals is limited. Often, the level of tolerance by the body is high, so they can be engineered to manage immune disorders like autoimmune diseases (Kori *et al*., 2009).

2.6.3 Anticancer property

A CG methanolic extract administration at 200 and 400 mg/kg of body weight intraperitoneal doses exhibited anticancer activity in the Ehrlich Ascites Carcinoma cell line. The results showed a significant reduction in tumour weight, viable cell count, tumour volume, and increased lifespan of the mice with tumours compared to the normal control group. The count of lymphocytes, white blood cells, red blood cells, and haemoglobin became normal in treated mice. The results were an indication of a dose-dependent anticancer effect comparable to 20 mg of 5-fluorouracil per kilogram body weight (Bala *et al.,* 2010). Observation of the lifespan of the mice and white blood cell count function as reliable approaches to evaluating anticancer remedies. Also, the reduction of tumour size points to a limitation of the abnormal cell division process.

The methanolic extract's ability to improve red blood cell count and haemoglobin concentration (Bala *et al*., 2010) differentiates it from a procedure like chemotherapy, often accompanied by anaemia due to a low red blood cell count. Phytochemical examinations that preceded this study identified alkaloids, tannins, and flavonoids as being present in the *C. gynandra* methanolic extract (Fotsis *et al*., 1997). Flavonoids can be anticancer and anti-mutagenic since they can influence signal transduction in cell growth and the formation of blood vessels (Bala *et al*., 2010).

2.6.4 Anti-microbial property

The anti-microbial effect of *C.gynandra* has been reported against different strains of microbes based on antimicrobial assays. The screening of *C. gynandra* extracts against

various gram-negative and gram-positive bacteria demonstrated variations in inhibition against the bacterial strains. The highly inhibited bacteria were *Streptococcus faecalis* and *Bacillus subtilis* compared to *Escherichia coli* and *Pseudomonas aeruginosa* which are resistant bacterial strains. The antibacterial activity is dependent on both the extraction solvent and part of the plant (Ajaiyeoba *et al*., 2001). As observed by (Sridhar *et al*., 2014), *C. gynandra* methanolic extract possesses antibacterial activity against *Staphylococcus aureus,* but different strains respond differently. In an attempt to test the antifungal potential of *C. gynandra, Candida albicans* was resistant to *C.gynandra* methanol extract (Hamil *et al*., 2003).

2.6.5 Anti-inflammatory property

The scientific demonstration of the anti-inflammatory action of *C. gynandra* involved thermal stimulus (a hot plate test) and the animal's reaction after an intraperitoneal infusion. The antinociceptive activity during the writhing response and hot plate tests had similarities with the standard use of naxolone (1 mg kg(-1), s.c.) (Ghogare *et al.,* 2009). These effects can be associated with flavonoids like bio-flavonoids and quercetin (Adhikari & Paul, 2018). By measuring the paw volume and the ethanolic extract's ability to maintain plasma and lysosome enzyme activities, Narenthirakannan *et al*. (2007) showed the possible mechanism of action of the extract in adjuvant-induced arthritis in rats, which is by stabilizing the lysosomal membrane (Narendhirakannan *et al.*, 2007). In the arthritic condition, the activity of the lysosomal enzymes was high. Though it was markedly reduced after the administration of the methanolic extract.

Modification of the lysosomal membrane may involve an interaction with the plasma membrane in a manner that limits the release of lysosomal enzymes or inhibits their release (Carević & Djokić, 1988). Also, the reduction in the enzyme activities inhibition

of the hydrolytic enzymes by triterpenes and flavonoids present in the *C. gynandra* leaf extract (Havsteen, 1983**;** Narendhirakannan *et al*., 2007**)**. Since glycoproteins, carbohydrate-protein macromolecules, are a major component of the cell membrane their reduced concentration in presence of the leaf extract may signify the stability of the lysosomal membrane. The change in the glycoprotein metabolism was linked to the increased release of lysosomal hydrolytic enzymes in an arthritic condition (Giuliani *et al*., 2002). In cases of arthritis, there is the movement of macrophages, lymphocytes, and polymorphonuclear leukocytes into the affected joints, where they produce cytokines. Therefore, limiting this movement can help limit inflammatory reactions at the joint, which was possible with *C. gynandra* leaf extract since flavonoids can curb leukocytes migration into the joint fluid and synovium (Damre *et al.*, 2003; Narendhikannan *et al*., 2007).

The progression of rheumatoid arthritis involves a complex interaction of cytokines. During the inflammations, cytokines such as interleukin-1, interleukin-6 and tumour necrosis factor-alpha (TNF-α) are present in the synovium where they are linked to apoptosis, joint injury, and growth of the synovial tissue (Choy & Panayi, 2001; Yeom *et al.,* 2003). Also, the expression of the inflammatory cytokines is increased. A cytokine-like TNF-α can bring about the stimulation of osteoclast differentiation, promote endothelial adhesion molecules, and promote the synthesis of other cytokines (Bazzoni & Beutler, 1996). Therefore, TNF-α factor can be more effective compared to other cytokines in the management of inflammation, and therapeutic agents capable of inhibiting the effects of the inflammatory cytokines can function as a rheumatoid arthritis treatment.

2.6.6 Anti-oxidant property

In cases of oxidative stress, the chemical diversity of plants with antioxidant properties can be beneficial. Due to the presence of vitamin C, oleic acid, stearic acid, and gamma-linoleic acid in *C. gynandra* leaves and other beneficial plants (Alam, 2007), the formation of free radicals can be limited (Narendhirakannan *et al.*, 2005). *In vitro,* an aqueous methanolic *C. gynandra* extract exhibited a high inhibitory effect during an antioxidant screening. Through a High-Performance Liquid Chromatography (HPLC) analysis a variation in the different phenolic compounds having different effectivenesses as antioxidants is shown (Ismail *et al*., 2004; Muchuweti *et al*., 2007). A compound like caffeic acid exhibits good antioxidant activities. The key to their higher effectiveness is the existence of the -CH=CH-COOH group, in comparison to the carboxyl functional groups (Kim *et al*., 2006). Flavonoids (present in *C. gynandra*) also have high antioxidant activity, with more hydroxyl groups compared to ferulic acids (Muchuweti et al., 2007). Moreover, flavonoid electron-donating methoxy or alkyl groups promote free-radicals' stability, hence their antioxidant property (Ismail *et al*., 2004).

2.5.2 Use of plant extracts

Likewise, plant extracts have shown effectiveness in managing mercuric chloride-induced nephrotoxicity. As shown in Table 1, studies with *Juglan Sinensis* extract (Beohm *et al.*, 2002), Silymarin – a flavonolignan mixture from *Silybum marianum* (Oda & El-Ashmawy, 2012), and ethanolic extract from *Eruca sativa* (Alam, 2007) containing glucoerucin and flavonoids as the significant antioxidants indicate that they possess renal protective activity from oxidative damage.

Prophylaxis with *Juglan sinensis* extract is capable of limiting acute renal injury due to mercuric chloride administration. As seen in experimental rabbits, the glomerular

filtration rate and urine quantity were significantly maintained compared to the positive control. Other measures of kidney injury, such as sodium ions fractional excretion and serum creatinine concentration, also presented a similar pattern. Additionally, the *Juglan sinensis* extract limited the uptake of *p*-Aminohippurate (PAH) by cortical tissue sections of the kidney (Beohm *et al.*, 2002). Since Ohsungi et al. (1999) demonstrated its scavenging activity against hydroxyl and superoxide anion radicals, the reduction of lipid peroxidation by the extract points to an antioxidant action.

Based on an experimental animal study, silymarin, an extract from *Silybum marianum*, can attenuate the toxic effects of mercuric chloride. Concentrations of malondialdehyde (MDA) and reduced glutathione were reduced to normal indices, attributable to the extract's ability to scavenge free radicals, hence antioxidant activity (Oda & El-Ashmawy, 2012). For the histological examinations, the intensity of the renal lesions was reduced, as supported by a concurrent reduction in the improvement of kidney function. The reduction in the activity of enzymatic markers including aspartate aminotransferase, alkaline phosphatase, and alanine aminotransferase indicates the lessened tissue injury through maintenance of the cellular membrane integrity, preventing their release into extracellular spaces (Pradeep *et al.*, 2007).

Table 1: A summary of plant extracts shown to have marked antioxidant activity against mercury chloride-induced nephrotoxicity

Plant	Plant extract	Active chemical components	Effects
Eruca sativa (Alam, 2007)	Ethanolic seed extract	- Glucoerucin - Flavonoids	- Potent antioxidant and kidney protective activity (protected against changes in the diagnostic parameters)
Silybum marianum (Oda & El-	Silymarin	- Flavonolignans	- Improved kidney function

Ashmawy, 2012)			
Juglan sinensis (Beohm *et al.*, 2002)	*Juglan sinensis*, Dode, hot water extract		- Prevents irreversible cell injury in the renal cortex.
Gingko biloba (Şener *et al.*, 2007)	Acetone/ water leaf extract	-Flavone glycosides -Terpenoids (ginkgolides).	- Restoration of biochemical changes brought by mercury intoxication.

Eruca sativa seed extract has a nephroprotective property against mercuric chloride, which is linked to its antioxidant activity (Alam, 2007). Phenolic compounds are often associated with free radical scavenging and the presence of high phenols quantity and reducing power is essential to its antioxidant potential (Barillari *et al.*, 2005); glucoerucin being the primary antioxidant (Alam, 2007). The presence of this antioxidant implicates the scavenging activity of free radicals and the induction of phase-two metabolizing enzymes (Zhang *et al.*, 1992). Being a glucosinolate, glucosinolates are often converted into isothiocyanates that activate phase two metabolizing enzymes, including Glutathione-S-transferase, essential for electrophile detoxification and limiting oxidative stress (Fahey & Talalay, 1999). Therefore, the extract can effectively curb reactive oxygen species and reactive nitrogen species. Such products are often toxic and negatively affect the structure and function of biomolecules.

The extract inhibited iron (III)-ascorbate-induced structural alterations on proteins and lipids. Iron (III) induces the formation of reactive oxygen species formation (Alam, 2007). Since there was no evidence of chelation, the protection can be attributed to the quenching of free radicals. Under conditions whereby the activities of catalase, glutathione peroxidase, and superoxide dismutase have been inhibited, the scavenging of hydrogen peroxide by the *Eruca sativa* seed extract may restore the activities of the

enzymes. Also, there is a potential for the induction of antioxidant system enzymes *de novo* synthesis.

Şener *et al*. (2007) designed an experimental study to determine the possible protection of *Gingko biloba* extract against mercury-induced damage to the brain, kidney, liver and lungs after an intraperitoneal administration of $HgCl_2$. The alterations were determined biochemically through chemical parameters. As markers of kidney dysfunction, creatinine ($p<0.001$) and BUN ($p<0.01$) were significantly increased on exposure to Hg, but the extract decreased their concentrations. Also in the presence of the extract marker of oxidative stress, that is, malondialdehyde and glutathione were maintained at a normal level. The increased malondialdehyde and decreased reduced glutathione implicate oxidative tissue injury that further caused functional impairment as shown by the kidney function tests (Şener *et al*., 2007). Due to tissue injuries generated by Hg neutrophils tend to infiltrate these tissues. As a result, the activity of myeloperoxidase, a marker of neutrophil tissue infiltration, was increased though the extract decreased its activity. Similarly, collagen content was decreased following increased fibrotic activity in the kidney tissue. These observations present it as an antioxidant agent that prevented oxidative tissue injuries and decreased functionality of the kidney.

These examples point to the ability of plant extracts to protect against mercury-induced kidney injuries.

CHAPTER THREE: METHODOLOGY

3.1. Study setting

The research took place in Eldoret, Uasin Gishu County, Kenya, from June 2021 to October 2021 in which all the relevant data were collected. The housing of the experimental animals was at Zoology lab 2, Department of Biological Sciences, University of Eldoret, Eldoret, Kenya. Within the same institution, at the Chemistry lab, Department of Chemistry and Biochemistry, the Crude extract of *Cleome gynandra* (CG) was obtained. Processing and analysis of the blood were achieved at the Department of Biochemistry and Clinical Chemistry Laboratory and Academic Model Providing Access to Healthcare (AMPATH) Reference Laboratory, respectively, located at the Moi Teaching and Referral Hospital (MTRH), Eldoret, Kenya. For histological studies, the examinations happened in the Department of Pathology, MTRH, Eldoret, Kenya.

The common denominator in selecting the research sites was the availability of the necessary resources essential for the attainment of the study aims. Since the limited research funds did not include the acquisition of the required equipment, the institutions needed to have existing analytical equipment.

3.2 Chemicals and reagents

Mercury in the form of mercuric chloride ($HgCl_2$) from Loba Chemie Pvt.Ltd, Mumbai, India (Product code: 0457400250) was purchased locally. Dimethyl sulphoxide was purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Methanol, Sodium Chloride, sodium chloride intravenous infusion (0.9% W/V), and formaldehyde solution were obtained from standard local suppliers.

3.3 Study design

It was an experimental animal study with a Completely Randomized Design.

3.4 Plant materials, sample preparation, and extraction

The cultivated fresh leafy vegetable, *Cleome gynandra*, was purchased from Kibuye (-0.093484, 34.768896) and Kiboswa (-0.024803, 34.747156) market places in Kisumu county, Kenya and botanically identified by a botanist from the University of Eldoret. The fresh leafy vegetable was packaged in hygienic non-woven carrier bags for transportation. The plant materials were cleaned using water and then shed-dried in a well-ventilated room for seven days. The dried plant material was packaged in a clean carton box, then sealed, for transportation. The weight of the dry plant material used was 350g. To obtain a homogeneous sample with an increased surface area for the extraction process, the leaves were grounded using a pestle and motor. Following this, was the extraction process through cold maceration (Pisoschi et al., 2016) using absolute methanol as the solvent, with frequent stirring for three days at room temperature. The ratio of the dry plant material to the solvent was 1:3. The damp mixture is pressed and the liquid was filtered using Whatman® filter papers. The extraction was repeated on the same sample. A concentrate of the filtered extract was then obtained by rotary evaporation at 40°C. Afterwards, further drying was done in an oven at 37°C producing a solid extract that was then dissolved in 10% DMSO (dimethyl sulphoxide) before oral administration. The percentage yield was 5.66%.

Determination of the percentage yield:

$$Percentage\ yield(\%) = \frac{Weight\ of\ the\ extract\ (g)}{Weight\ of\ the\ dry\ plant\ material\ (g)} \times 100$$

$$= \frac{19.82}{350} \times 100$$

$$= 5.66\ \%$$

3.5 Sample size determination

The Resource Equation method was applied in the determination of the sample size (Charan & Kantharia, 2013) due to the multiple variables measured and the inability to determine the effect size. It was only applicable in the first objective.

E = Total number of animals – Total number of groups

Where E is the degree of freedom of ANOVA. A value of E between 10 and 20, indicates adequacy in sample size. For an E value below 10, more animals are required to maximize the probability of getting more significant outcomes. However, if the E value is above 20, increasing the number of experimental animals will not improve the probability of obtaining results which are significant. This study had an E of 16. For the first objective, there were four groups, with each group having five rats, hence the total number of animals = 5×4 = 20.

In the evaluation of the second objective ten experimental animals were used.

3.6 Experimental animals

A total of thirty male albino Wistar rats, 2 months of age, 180-220 g in weight, were procured from the University of Eldoret, Kenya, and placed in the Department of Zoology animal house, University of Eldoret. They were housed in wire cages with wood chips and soft grass laid at the base of the cage (5 rats in each cage) and allowed to acclimatize to the environment for one week before the experiment. Throughout the study, the rats were allowed to freely access food (standard rat pellets) and water. They were maintained in a well-ventilated and clean environment, under room temperature.

The rats were randomly divided into groups of five as follows:

Grouping for the first objective.

Group 1 (n=5) - This was a vehicle control group. Rats in this group received an oral administration of 1 ml of 10% dimethyl sulfoxide (DMSO) solution daily and a

subcutaneous injection of 0.5 ml normal saline on the 7th day 2 hours after the last dose of 10% DMSO solution.

Group 2 (n=5) - This was the $HgCl_2$-treated (negative control) group that received a daily oral administration of 1 ml 10% DMSO solution and subcutaneous injection of 5 mg of mercuric chloride ($HgCl_2$) per kg body weight (bwt) in 0.5 ml normal saline solution on the 7th day 2 hours after the last dose of 10% DMSO.

Group 3 (n=5) - This was the CG+$HgCl_2$-treated(D1) group, which received a daily oral administration of 250 mg of the extract per kg body weight in 1 ml 10% DMSO solution and a subcutaneous injection of 5 mg of $HgCl_2$ per kg bwt in 0.5 ml normal saline on the 7th day 2 hours after the last dose of extract in 10% DMSO solution.

Group 4 (n=5) - This was the CG+$HgCl_2$-treated(D2) group. The experimental rats received a daily oral administration of 500 mg of the extract per kg body weight in 1 ml 10% DMSO solution and a subcutaneous injection of 5 mg of $HgCl_2$ per kg bwt in 0.5 ml normal saline on the 7th day, 2 hours after the last dose of extract in 10% DMSO solution.

Grouping for the second objective.

Group 5 (n=5) - The rats in a $HgCl_2$-treated (0.5mg/kg bwt) (negative control) group (n=5) received a daily oral administration of 1 ml 10% DMSO solution and a daily subcutaneous injection of 0.5 mg of $HgCl_2$ per kg bwt 2 hours later. In this group, a random rat was sacrificed 24 hours after the 1st, 4th, and 7th days.

Group 6 (n=5) - The rat in a CG+$HgCl_2$-treated group (n=5) received a daily oral administration of 200 mg of CG extract in 1 ml 10% DMSO and 0.5 mg $HgCl_2$/kg bwt in normal saline daily subcutaneous injection two hours later. In this group, a random rat was sacrificed 24 hours after the 1st, 4th, and 7th days.

3.7 Collection and processing of blood and kidney samples

Twenty-four hours after the last mercuric chloride administration, blood and tissue samples were collected. In groups 1 to 4, after euthanizing the rats with chloroform, at least 5 ml of blood were collected from the heart through cardiac puncture into ethylenediaminetetracetic acid (EDTA) laced vacutainers and mixed gently. Blood was then centrifuged at 3,500 revolutions per minute for 15 minutes to obtain plasma and transferred into cryogenic vials in preparation for the biochemical analyses. In groups 1 to 6, using sterilized surgical blades, kidney pairs were isolated from the surrounding tissues after dissection on a dissection tray and pat dried using dry Whatman® filter papers. The kidney pairs were then fixed by submerging in 10% formalin in polypropiolactone transparent plastic tissue containers for preservation. The kidneys were used for histopathological examinations.

3.8 Biochemical assays

3.8.1 Kidney function indices

Photometric quantitative analysis of plasma albumin, urea, and creatinine was done using COBAS INTEGRA® 400 plus auto-analyzer from Roche Company at Moi Teaching and Referral Hospital (MTRH), Eldoret, Kenya. Blood urea and creatinine rise in cases of renal injury, primarily indicate decreased functionality of glomerular filtration. An increase in plasma creatinine concentration usually follows the increase in plasma urea level in the initial renal damage stages. The plasma urea levels are dependent on factors such as hydration, diet, and protein metabolism; however, plasma creatinine level is independent of these factors. Serum or plasma albumin level can also be an indicator of kidney function in combination with creatinine and urea.

The concentration units in the analysis reports of albumin, urea, and creatinine were g/L, µmol/L, and mmol/L, respectively. The units for albumin, urea, and creatinine

were converted to g/dL, mg/dL, and mg/dL which are commonly used in most literature. Below are the conversion criteria:

Conversion of g/L to g/dL of albumin - Division by 10.

Conversion of µmol/L to mg/dL creatinine - Multiplication by 0.0113.

Conversion of mmol/L to mg/dL urea - Division by 0.3571.

3.9 Histological examinations

They were performed on the kidney samples of the animal groups 1 to 6. Tissues from the samples were fixed for 72 hours in 10% formalin (in phosphate buffer – pH 7). Afterwards, they were embedded in paraffin wax. 5-micrometer sections were cut using a rotary microtome, transferred to microscope slides, and stained using hematoxylin-eosin for light microscopy. The observations were recorded primarily by semi-quantitatively estimating the relative severity of tissue alterations. For consistency, observations were made on the glomerulus and the Bowman's capsule for groups 5 and 6, and the glomerulus, Bowman's capsule, and proximal tubule for groups 1 to 4. Being that this process involves estimations rather than precise measurements, it is considered semi-quantitative.

3.10 Data management plan

3.10.1 Data entry and cleaning

The numerical biochemical parameters measurements data were entered into the Microsoft Excel Spread Sheet and cleaned by checking and correcting typing errors, miscodes, and entry errors.

3.10.2 Data analysis

The entered quantitative data were statistically analyzed with the GraphPad Prism software. A check for outliers using Grubbs' test or the Extreme Studentized Deviate method indicated no outliers. The data were expressed as mean ± standard deviation.

Using one-way analysis of variance with the Tukey post hoc test the statistical comparisons for all the parameters were done. The statistical significance was set at $P \leq 0.05$.

The relative severity of the injuries was recorded semi-quantitatively. The semi-quantitative analysis involved the use of defined ranges of severity or severity grades for the specific injuries (Hackelford et al., 2002). The grading scheme for the severity grading had four severity grades, namely, grade 1 (minimal), grade 2 (mild), grade 3 (moderate), and grade 4 (marked). Grade 1 corresponded to histological alteration that may be hardly distinguishable to minor alterations (0 - 25% of the tissue is altered). In grade 2 the histological change is distinguishable, however, it is not a main characteristic of the tissue (26-50% of the tissue is affected). The histological change in grade 3 is the prominent feature of the tissue where 51-75% of the tissue is altered. Grade 4 relates to a histological change which is the overwhelming characteristic of the tissue involving 76-100% of the tissue.

3.10.3 Data representation

The data from the biochemical studies are presented in tables with groups on the horizontal axis and the means on the vertical axis for each biochemical parameter. For histopathological studies, the visual data are in the form of photomicrographs. A random photomicrograph in each of groups 1 to 4 was used to represent the findings, while in group 5 and 6 the photomicrographs were of all the sacrificed rats.

3.11 Ethical considerations

The study was approved by the University of East Africa Baraton Ethics Committee (UEAB/REC/04/05/2021) (Appendix 1). Guidelines in the guide for laboratory animals' care were followed during the study (NRC, 2010).

CHAPTER FOUR: RESULTS

This chapter represents findings on the protective effects of *Cleome gynandra* CG extract on the kidney exposed to mercury chloride acutely, where the levels of the biochemical parameters are in tables and photomicrographs represent histological changes data in the kidney. In section 4.1, the focus is on the protective effects of the CG extract against the damaging effects of a high dose of $HgCl_2$ (5 mg/kg bwt) on the kidney, while the second section (section 4.2) is on the CG extract protective effects against the effects of a low dose of $HgCl_2$ (0.5 mg/kg bwt) on the kidney. The raw measured data for the biochemical parameters are in *appendix 2*.

4.1 Effects of *Cleome gynandra* (CG) leaf extract on the kidney exposed to a probable lethal dose of $HgCl_2$ (5 mg/kg bwt)

4.1.1 Biochemical parameters

A summary of the results of the first objective on the biochemical parameters of kidney function is presented in the table below (table 2). Twenty blood samples from four groups of the experimental rats were analyzed to determine the concentrations of blood creatinine, urea, and albumin. A single mercuric chloride dose (5 mg/kg bwt) administered subcutaneously brought about renal toxicity, exhibited by the significant biochemical increase in blood plasma urea (p=0.021) and creatinine (p=0.032) in table 6 and 4, respectively. In comparison to the vehicle control, creatinine and urea levels were significantly increased in CG+$HgCl_2$-treated(D1) (p=0.000 and p=0.000, respectively) and CG+$HgCl_2$-treated(D2) (p=0.001 and p=0.003, respectively) groups as indicated in tables 4 and 6. Unlike other blood biochemical parameters, albumin concentrations were lower in the CG extract treated groups compared to the controls. Significance (p=0.02) was in the group receiving the highest concentration of CG extract (500mg/kg body weight) compared to the vehicle control group. Though there

were differences between CG+$HgCl_2$-treated(D1) and CG+$HgCl_2$-treated(D2), the differences were insignificant for creatinine (p=0.972), urea (p=0.341), and albumin (p=0.254).

Table 2: Levels of urea (mg/dL), albumin (g/dL), and creatinine (mg/dL) in the blood at the end of the experiment presented as the mean ± SD (n=5).

Groups	**Mean creatinine levels (mg/dL)**	**Mean urea levels (mg/dL)**	**Mean albumin levels (g/dL)**
Vehicle Control	0.294 ± 0.020	15.172±3.299	3.951 ± 0.422
$HgCl_2$-treated	1.907 ± 1.238[a]	78.482±49.595[a]	3.401 ± 0.232
CG+$HgCl_2$-treated(D1)	3.120 ± 0.291[a]	130.434±13.568[a]	3.011 ± 0.354
CG+$HgCl_2$-treated(D2)	2.893 ± 1.056[a]	97.149 ± 32.299[a]	2.117 ± 1.334[a]

Key:
[a] Significantly different from the vehicle control, $p \leq 0.05$
Vehicle control (n=5) – 10% DMSO + normal saline
$HgCl_2$-treated (n=5) – 10% DMSO + 5mg $HgCl_2$/kg bwt in 0.9%NaCl
CG+$HgCl_2$-treated(D1) (n=5) – 250mg CG extract/kg bwt + 5mg $HgCl_2$/kg bwt in 0.9% NaCl
CG+$HgCl_2$-treated(D2) (n=5) – 500mg CG extract/kg bwt + 5mg $HgCl_2$/kg bwt in 0.9%NaCl

4.1.1.1 Levels of blood creatinine

The concentrations of the blood creatinine for the twenty blood samples were determined using the COBAS Integra 400 plus auto-analyzer and the means and standard deviations calculated. The mean creatinine concentrations (±SD) in the vehicle control group (n=5), $HgCl_2$-treated group (n=5), CG+$HgCl_2$-treated group (D1) (n=5), and CG+$HgCl_2$-treated group (D2) (n=5) were 0.294±0.020, 1.907±1.238, 3.120±0.291, and 2.893±1.056 mg/dL (Table 2). Only the mean concentration in the vehicle control (0.294 mg/dL) was within the normal range of 0.2 - 0.7 mg/dL and the highest concentration was that of the CG+$HgCl_2$-treated(D1) group (3.120±0.291 mg/dL).

One-way Analysis of Variance (ANOVA) (*F ratio*=12.09, *p*=0.0002) presents that the statistical difference in the creatinine concentration between the 4 groups was significant, that is, the means differ highly as shown in table 3 below, by the F ratio (ratio of the mean squares) and *p*-value. However, this does not mean that every group's mean differs from any other mean, only that at least one differs from the rest. As represented by the R square (that is, 0.6939) in table 3, 69.39% of the variation is attributable to the treatments defining the experimental groups.

Table 3: One-way ANOVA summary of the effect of CG on the level of blood creatinine in mercury intoxicated rats *(p<0.05)*

One-way analysis of variance			
P value			0.0002
Are means significantly different? (P < 0.05)			Yes
Number of groups			4
F ratio			12.09
R square			0.6939
ANOVA Table	**Sum of Squares**	**Degree of Freedom**	**Mean Squares**
Treatment (between groups)	24.796	3	8.265
Residual (within groups)	10.937	16	0.684
Total	35.733	19	

In table 4, variability between the different groups is given. The Tukey post hoc test indicates that the creatinine concentration in the $HgCl_2$-treated (1.907±1.238 mg/dL)(*p*=0.032), CG+$HgCl_2$-treated(D1) (3.120±0.291 mg/dL)(*p*=0.000), and CG+$HgCl_2$-treated(D2) (2.893±1.056 mg/dL)(*p*=0.001) groups are significantly increased compared to the vehicle control (0.294±0.020 mg/dL). The increase observed

in CG+$HgCl_2$-treated(D1) (p=0.135) and CG+$HgCl_2$-treated(D2) (p=0.272) groups compared to the $HgCl_2$-treated group was statistically insignificant. Based on the q-value and mean difference, CG+$HgCl_2$-treated(D2) group shows a reduction in creatinine concentration compared to the CG+$HgCl_2$-treated(D1), however, the decrease is not significant (p=0.972).

Table 4: Multiple comparisons of mean blood creatinine levels between the four experimental groups *($p<0.05$)*

Tukey's Multiple Comparison Test	**Mean Diff.**	**q**	**95% CI of the diff**	**Sig. (P < 0.05)**
Vehicle Control vs. $HgCl_2$-treated	-1.613	4.362	-3.109 to -0.117	0.032
Vehicle Control vs. CG+$HgCl_2$-treated(D1)	-2.826	7.643	-4.322 to -1.330	0.000
Vehicle Control vs. CG+$HgCl_2$-treated(D2)	-2.599	7.029	-4.085 to -1.103	0.001
$HgCl_2$-treated vs. CG+$HgCl_2$-treated(D1)	-1.213	3.281	-2.704 to 0.282	0.135
$HgCl_2$-treated vs. CG+$HgCl_2$-treated(D2)	-0.986	2.667	-2.282 to 0.105	0.272
CG+$HgCl_2$-treated(D1) vs. CG+$HgCl_2$-treated(D2)	0.227	0.614	-1.268 to 1.723	0.972

4.1.1.2 Levels of blood urea

For the urea mean levels in table 2, The highest mean concentration was observed in CG+$HgCl_2$-treated(D1) group (130.434±13.568 mg/dL) while the lowest in the vehicle control group (15.172±3.299 mg/dL) which is within the normal range of 11 - 25 mg/dL. Between the highest and the lowest mean concentrations are the mean concentrations of the $HgCl_2$-treated group (78.482±49.595 mg/dL) and CG+$HgCl_2$-treated(D2) groups (97.149±32.299 mg/dL).

As determined by one-way ANOVA (F=12.70, p=0.0002) in table 5, there is a statistical difference in the means (as indicated by the p-value) in the blood urea concentration between the experimental groups. It is because the F ratio is greater than 1 and the p-value is less than 0.05. Hence, at least one group's mean differs from the other groups' means and the difference is high. The R square value in table 5 shows that 70.42% of the variation is due to the treatments defining the groups.

Table 5: One-way ANOVA summary of the effect of CG on the level of blood urea in mercury intoxicated rats *($p<0.05$)*

One-way analysis of variance			
P value			0.0002
Are means significantly different? (P < 0.05)			Yes
Number of groups			4
F			12.70
R square			0.7042
ANOVA Table	**Sum of Squares**	**Degree of Freedom**	**Mean Square**
Treatment (between groups)	35211.277	3	11737.092
Residual (within groups)	14791.216	16	924.451
Total	50002.492	19	

The results from the Tukey post hoc test in table 6 revealed that the urea concentration at the end of the experiment was statistically significantly higher in the $HgCl_2$-treated group at 78.482±49.595 mg/dL (p=0.021), CG+$HgCl_2$-treated(D1) group at 130.434±13.568 mg/dL (p=0.000), and CG+$HgCl_2$-treated(D2) group at 97.149 ±32.299 mg/dL (p=0.003) compared to the vehicle control group at 15.172±3.299 mg/dL. There was no statistically significant difference between the $HgCl_2$-treated

group and CG+$HgCl_2$-treated(D1) group (p=0.068) and between the $HgCl_2$-treated and CG+$HgCl_2$-treated(D2) group (p=0.768). There was no statistically significant difference between CG+$HgCl_2$-treated(D1) and CG+$HgCl_2$-treated(D2) groups (p = 0.341), even with the reduction in the blood urea concentration.

Table 6: Multiple comparisons of mean blood urea levels between the four experimental groups *($p<0.05$)*

Tukey's Multiple Comparison Test	**Mean Diff.**	**q**	**95% CI of the diff**	**Sig. (P < 0.05)**
Vehicle Control vs. $HgCl_2$-treated	-63.310	4.656	-118.3 to -8.3	0.021
Vehicle Control vs. CG+$HgCl_2$-treated(D1)	-115.261	8.477	-170.3 to -60.2	0.000
Vehicle Control vs. CG+$HgCl_2$-treated(D2)	-81.977	6.029	-137.0 to -27.0	0.003
$HgCl_2$-treated vs. CG+$HgCl_2$-treated(D1)	-51.952	3.821	-107.0 to 3.1	0.068
$HgCl_2$-treated vs. CG+$HgCl_2$-treated(D2)	-18.667	1.373	-73.7 to 36.4	0.768
CG+$HgCl_2$-treated(D1) vs. CG+$HgCl_2$-treated(D2)	33.285	2.448	-21.7 to 88.3	0.341

4.1.1.3 Levels of albumin in the blood

In table 2, the plasma albumin concentration for twenty experimental rats are represented with the means (±SD) of the respective animal groups. The highest mean albumin concentration was observed in the vehicle control (3.951±0.422 g/dL), followed by the $HgCl_2$-treated (3.401±0.232 g/dL) and CG+$HgCl_2$-treated(D1) (3.011±0.354 g/dL) groups, with the lowest mean concentration observed in the

CG+$HgCl_2$-treated(D2) group (2.117±1.334 g/dL). Only the mean concentrations of the vehicle and $HgCl_2$-treated groups were within the normal range of 3.1-4.6 g/dL.

Based on the one-way ANOVA (F=5.578, p=0.0082), there was a statistically significant difference between the groups as shown in table 7, with at least one differing group mean. About half of the variation is due to the treatments defining the groups as indicated by the R squared value in table 7.

Table 7: One way ANOVA summary of the effect of CG on the level of blood albumin in mercury intoxicated rats *(p<0.05)*

One-way analysis of variance	
P value	0.0082
Are means significantly different? (P < 0.05)	Yes
Number of groups	4
F	5.578
R square	0.5112

ANOVA Table	**Sum of Squares**	**Degree of Freedom**	**Mean Square**
Treatment (between groups)	8.937	3	2.979
Residual (within groups)	8.546	16	0.5341
Total	17.483	19	

According to the multiple comparisons (Tukey post hoc test) in table 8, the mean albumin level of 2.117±1.334 g/dL in CG+$HgCl_2$-treated(D2) group was significantly lower compared to the vehicle control mean level of 3.951±0.422 g/dL (p=0.005). For the vehicle control and the $HgCl_2$-treated groups (3.401±0.232 g/dL), the difference in reduction between the two groups was insignificant (p=0.641). A similar observation was made between CG+$HgCl_2$-treated(D2) and CG+$HgCl_2$-treated(D1) group

(3.011±0.354 g/dL) (p=0.254), vehicle control and CG+$HgCl_2$-treated(D1) group (p = 0.216), and $HgCl_2$-treated and CG+$HgCl_2$-treated(D2) group (p=0.059), where the differences were insignificant. Also decrease in albumin levels between the $HgCl_2$-treated and CG+$HgCl_2$-treated(D1) group was insignificant (p=0.832).

Table 8: Multiple comparisons of mean blood albumin levels between the four experimental groups *($p<0.05$)*

Tukey's Multiple Comparison Test	**Mean Diff.**	**q**	**95% CI of diff**	**Sig. (P < 0.05)**
Vehicle Control vs. $HgCl_2$-treated	0.550	1.683	-0.772 to 1.873	0.641
Vehicle Control vs. CG+$HgCl_2$-treated(D1)	0.941	2.878	-0.382 to 2.263	0.216
Vehicle Control vs. CG+$HgCl_2$-treated(D2)	1.834	5.611	0.512 to 3.156	0.005
$HgCl_2$-treated vs. CG+$HgCl_2$-treated(D1)	0.391	1.195	-0.932 to 1.713	0.832
$HgCl_2$-treated vs. CG+$HgCl_2$-treated(D2)	1.284	3.928	-0.039 to 2.606	0.059
CG+$HgCl_2$-treated(D1) vs. CG+$HgCl_2$-treated(D2)	0.893	2.733	-0.429 to 2.216	0.254

4.1.2 Histological Changes

The results from the pathological examinations functioned as a supplement to the quantitative data on the biochemical parameters. The alterations varied in the various animal groups ranging from normal morphology to extensive alterations at the end of the experiment period among the groups. The figures below show photomicrographs of kidney tissues stained using hematoxylin-eosin.

As expected, the vehicle control group exhibited normal morphology of the parenchyma tissue of the kidney. There were no visible lesions and the glomeruli and tubules had well-defined structures (figure 3).

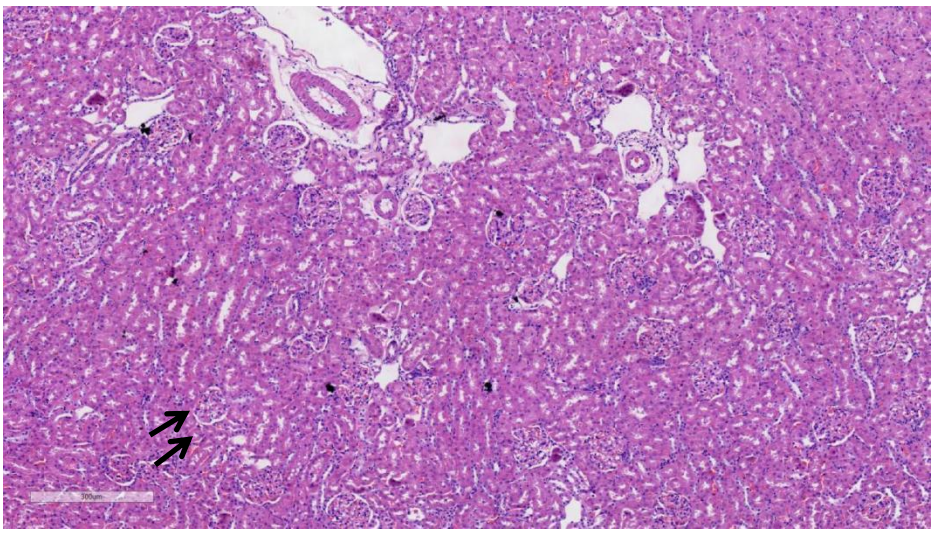

Figure 3: A photomicrograph of a kidney section showing the renal cortex of a vehicle control rat. The arrows are indicating normal tubule and glomeruli.

Unlike the vehicle control group, the $HgCl_2$-treated group, which received only $HgCl_2$, showed extensive tubular and glomerular damages (figure 4). The Bowman capsule was dilated and the glomerulus exhibited atrophy and instances of swelling. Also, a report of tubular necrosis in the damaged areas accompanied the observation of glomerular degeneration.

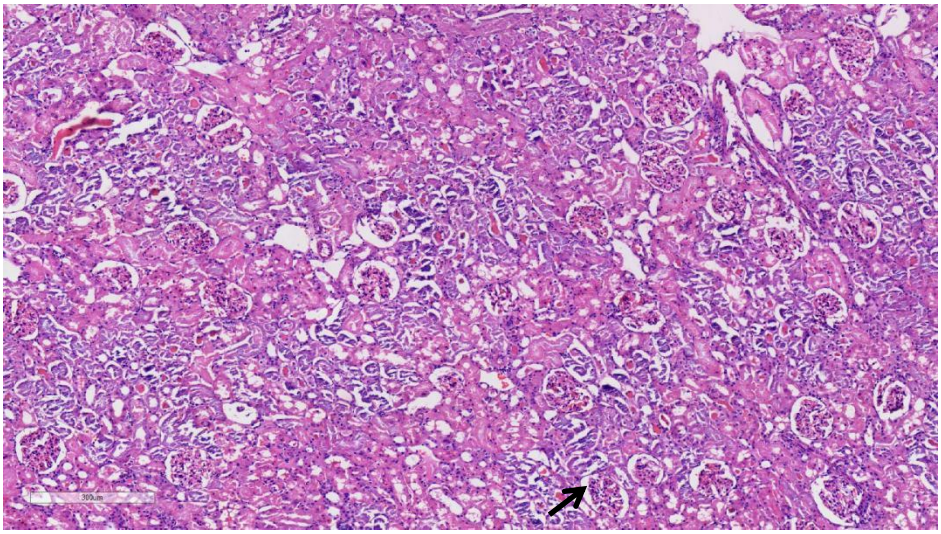

Figure 4: A photomicrograph of a kidney section of mercuric chloride treated rats. The arrows are showing degenerative changes.

In the CG+$HgCl_2$-treated(D1) group receiving 5 mg per kg body weight $HgCl_2$ and *C. gynandra* (CG) extract (250 mg per kg body weight), damage on the glomeruli was moderate while the tubular damage was extensive (figure 5). Also dilation of the Bowman's capsule and tubular necrosis was observed. However, the glomeruli were swollen.

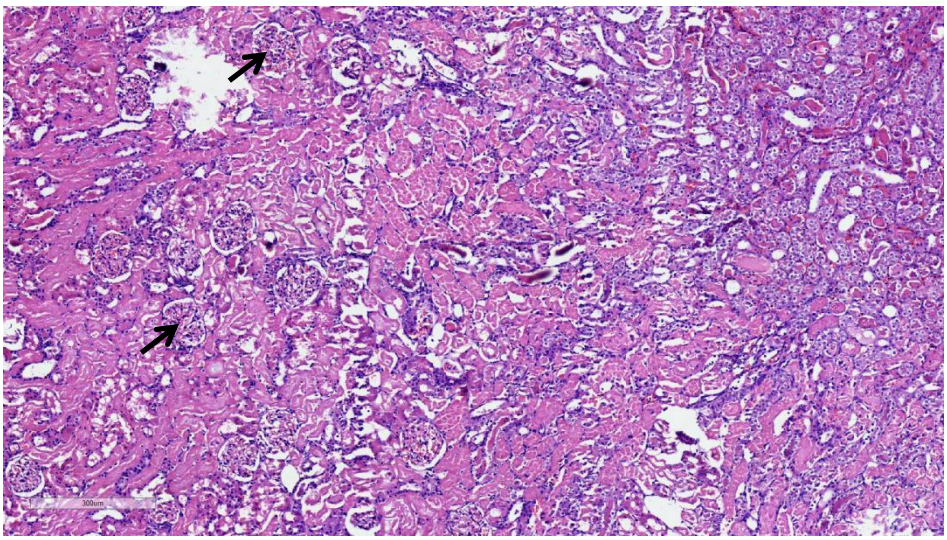

Figure 5: A photomicrograph of a kidney section of CG (250 mg/kg bwt) extract and mercuric chloride treated rat; the arrows showing degenerative alterations.

In CG+$HgCl_2$-treated(D2) group as shown in figure 6 below, both the glomeruli and the tubules were affected. With the presence of $HgCl_2$ and 500mg per kg body weight, the damage on the glomeruli and tubules was extensive as it was a prominent feature of the tissue. There was dilation of the Bowman's capsule and swelling of the glomeruli with tubular necrosis in degenerative instances of tissue alteration. Unaffected tissues showed normal physiology.

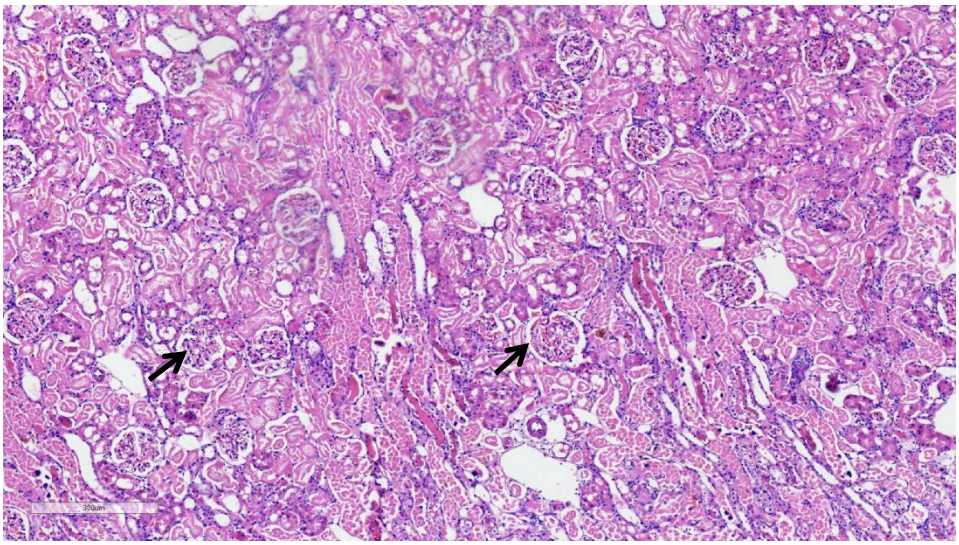

Figure 6: A photomicrograph of a kidney section of CG extract (500mg/kg bwt) and mercuric chloride exposed rats. The arrows are showing evidence of degenerative changes.

4.2. Effects of *Cleome gynandra* (CG) leaf extract on the kidney exposed to a low dose of $HgCl_2$

4.2.1 Histological changes

In this section, the pathological alterations related to the protective effects of the *C. gynandra* (CG) extract against the effects of a low mercury dose are recorded. Based on the light microscopy evaluation, the $HgCl_2$-treated group (group 5) and the CG+$HgCl_2$-treated group (group 6) showed varying observations with the progression of the days as shown in figures 7-12.

On day 2 as shown figures 7 and 8, the kidney evaluation of both the $HgCl_2$-treated (group 5) and CG+$HgCl_2$-treated group (group 6) showed normal morphology with well-defined glomeruli with barely noticeable changes that were infrequent.

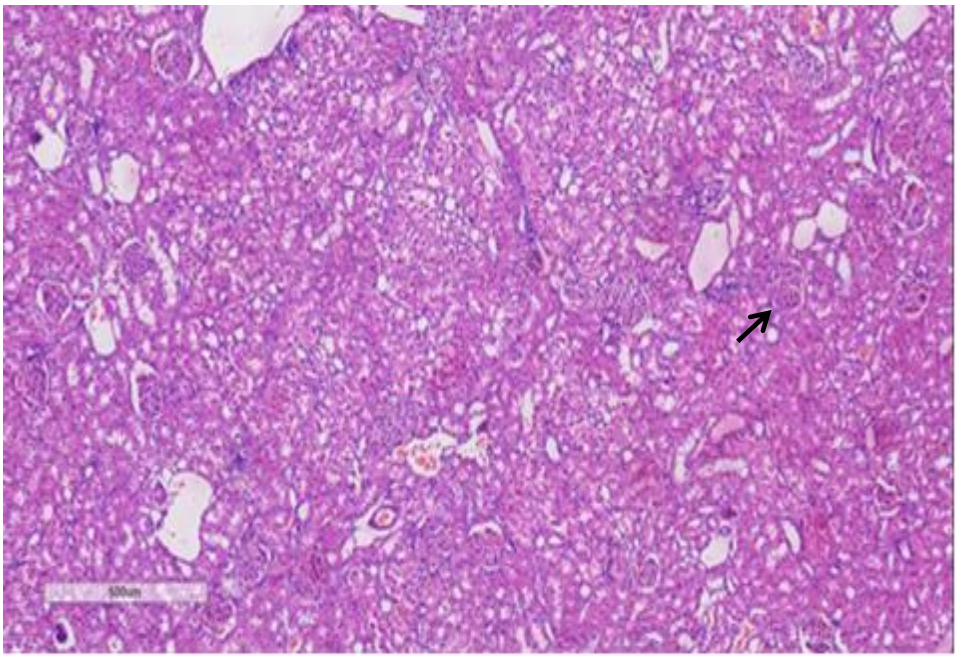

Figure 7: Day 2 photomicrograph of the renal cortex of the negative control group receiving $HgCl_2$. The arrow showing degenerative features on the glomeruli.

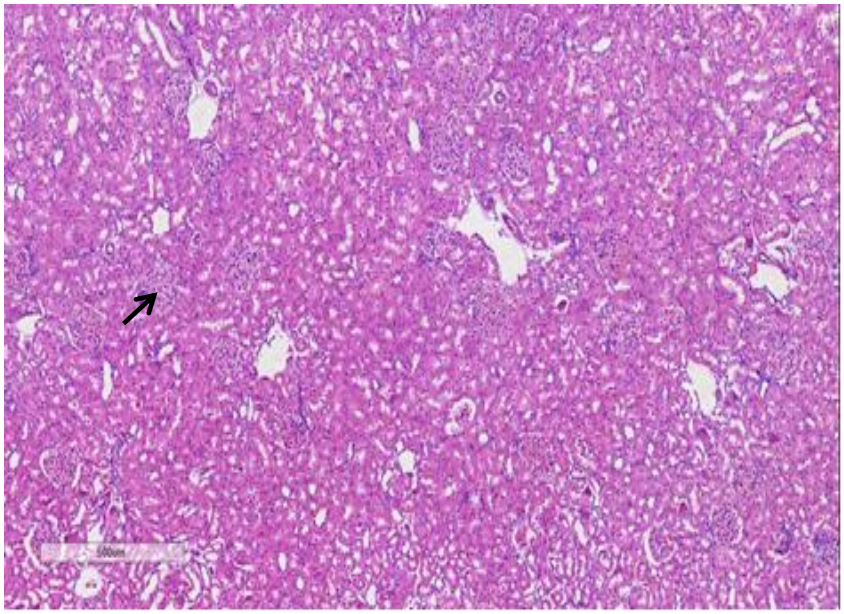

Figure 8: Day 2 photomicrograph of the renal cortex of the treatment group receiving $HgCl_2$ and CG extract. The arrow showing degenerative features on the glomeruli.

Similar observations compared to day 2 were made on the CG+$HgCl_2$-treated group (figure 10) on the 5^{th} day, where the changes were minimal (12.5%). In the $HgCl_2$-treated group (figure 7), the morphological changes were moderate (75%) indicating the prominence of the tissue changes. There was inflammation of the glomeruli with dilation of the Bowman capsule (figure 9).

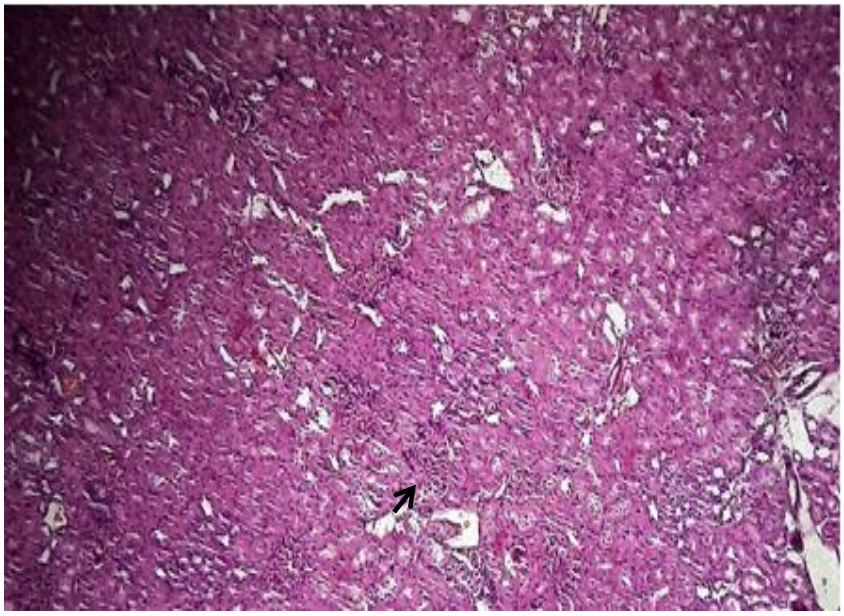

Figure 9: Day 5 photomicrographs of the renal cortex of the negative control group receiving $HgCl_2$. The arrows showing signs of degenerative features on the glomeruli.

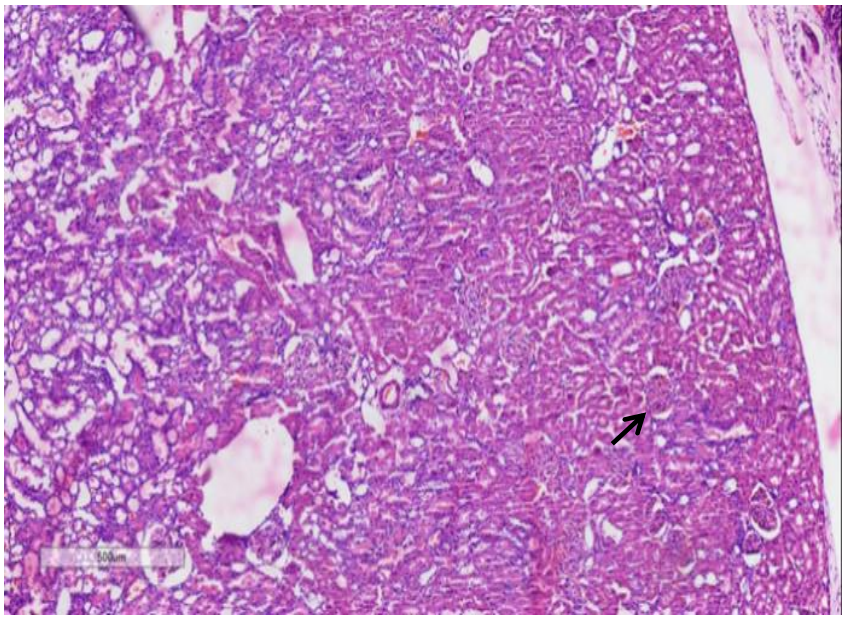

Figure 10: Day 5 photomicrographs of the renal cortex of the treatment group receiving $HgCl_2$ and CG extract. The arrow showing degenerative features on the glomeruli.

Observations made on the CG+$HgCl_2$-treated group on the 8th day (figure 12) showed minimal changes (12.5%). However, extensive or marked changes of approximately 100%, showing it as an extensive feature of the tissues, were observed in the $HgCl_2$-

treated group, figure 11, on the 8th day. The glomeruli showed degenerative features with inflammation and the Bowman's capsule exhibited dilation.

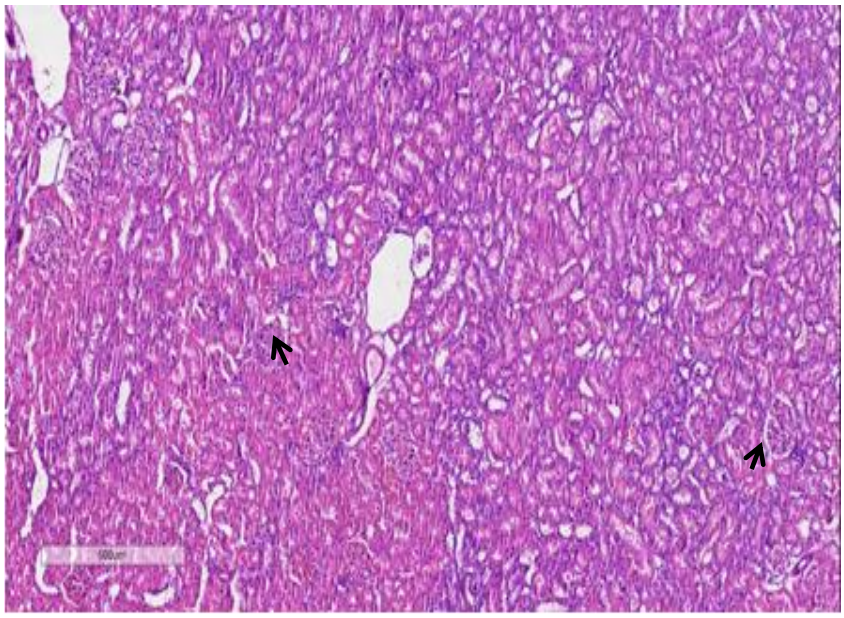

Figure 11: Day 8 photomicrographs of the renal cortex of the negative control animal group receiving $HgCl_2$. The arrows are showing signs of degenerative features on the glomeruli.

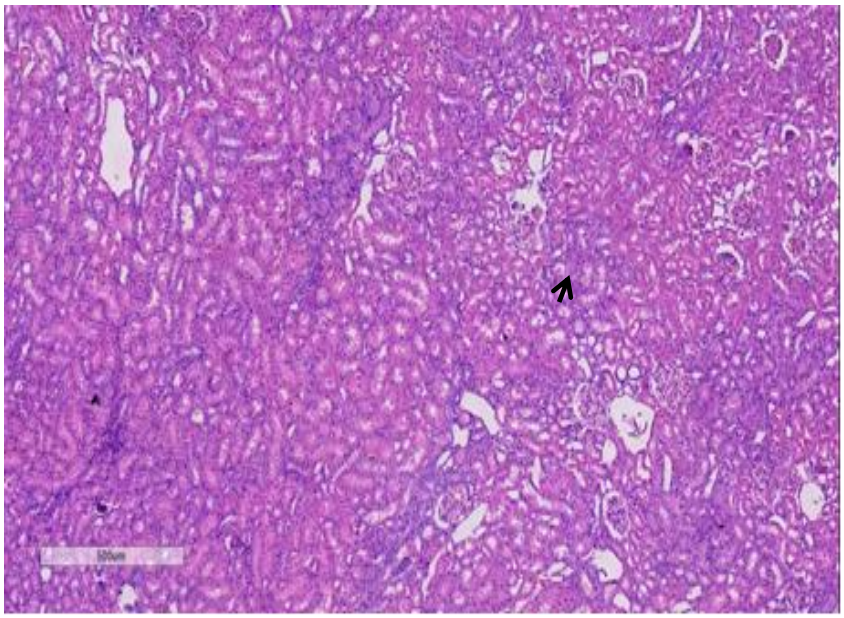

Figure 12: Day 8 photomicrographs of the renal cortex of the treatment animal group receiving $HgCl_2$ and CG extract. The arrow showing degenerative features on the glomeruli.

Generally, treatment with CG extract limited the progression of the degenerative alterations to the minimum compared to the $HgCl_2$-treated that showed increased degenerative alterations with the progression of the days.

CHAPTER FIVE: DISCUSSION

This work investigated the protective effects of *Cleome gynandra* (CG) against a mercuric chloride - induced renal injury in male Wistar albino rats. The investigation was through examination of kidney function by determining blood plasma creatinine, urea, and albumin concentrations and histopathological examinations on the renal cortex of the experimental subjects.

5.1 Effects of *Cleome gynandra* leaf extract on the kidney exposed to a probable lethal dose of $HgCl_2$ (5 mg/kg bwt)

The results from the current study showed no significant improvement in the kidney function of the experimental rats exposed acutely to a high dose of mercuric chloride ($HgCl_2$), that is, 5 mg per kilogram body weight. This observation shows that pretreatment with the *Cleome gynandra* (CG) extract, orally, before mercury exposure may be insufficient to protect against acute mercury-induced kidney injury. It is due to the extract's inability to maintain the blood creatinine, urea, and albumin at normal levels, as indicated in tables 2, respectively. The table contains measured mean concentrations of the biochemical parameters. For the biochemical parameters, blood urea and creatinine concentrations in the vehicle control were within the physiologically normal range of rats (11 - 25 mg/dL and 0.2 - 0.7 mg/dL, respectively), except for the albumin concentrations whereby both the vehicle control and $HgCl_2$-treated groups' albumin levels were within the normal range (3.1 - 4.6 g/dL). However, these observations are not conclusive of exposure to other levels of mercury concentration. The histological changes seen in the kidney back up the blood biochemical parameters. In the current study, a significant rise in blood creatinine levels (p=0.032) was noted in the $HgCl_2$-treated group exposed to $HgCl_2$ (table 4). Other studies have also reported a

similar finding, such as that by Gado *et al.* (2014) and Alam (2007) where mercuric chloride administration was intraperitoneal and subcutaneously, respectively. A rise in the level of creatinine often signifies cases of renal injury, primarily, a decline in the functionality of the glomerular filtration (Zalup, 2000), which in this study is evidenced by degenerative damages to the glomeruli including Bowman's capsule dilation and glomerular atrophy (figure 4). Since the mechanism for the reduction in glomerular filtration is not defined in this study, it gives room for the assumption that multiple factors could be involved in a likely complex process including Bowman's capsule dilation and glomerular atrophy. Also, since the proximal tubules excrete creatinine, the increased concentration of the creatinine can, to a lesser extent, indicate damage to the proximal tubules (Zalup, 2000) as shown by the tubular damage with tubular necrosis in the $HgCl_2$-treated group of the current study. These changes point to a decreased number of functional nephrons similar to when kidneys are surgically reduced in experimental rats (Zalup, 2000). Since creatinine is less susceptible to non-renal factors it is a sensitive indicator of renal function, hence, its measurement is a good index of renal function.

As a biomarker, creatinine is relatively insensitive to minor injuries to the kidney (Waikar & Bonventre, 2009). Since the reported results in the current study show a significant increase in the creatinine concentration, the pathological damage to the kidney tissue structure due to $HgCl_2$ exposure cannot be considered minor, as evidenced by the extensive tubular and glomerular damage (figure 4) that are tissue injury indicators validating the creatinine concentration. Similar histological observations were observed in a study focusing on the chronic effects of mercury inhalation on the rat kidney. The tubules displayed necrosis and the glomeruli were degenerated. This is

to show that ultimately both the elemental mercury and inorganic mercuric salts bring about the same effects (Akgül *et al.*, 2016).

The increase in creatinine level was 1.613 mg/dL, which is considered acute since the creatinine increase was more than 0.3 mg/dL within two days (Waikar & Bonventre, 2009). An increase of more than 0.3 mg/dL was also indicated by Abarikwu *et al.* (2017), that is, 2.018 mg/dL. However, the mercury administration was intra-peritoneal at a 5 mg/ kg body weight of mercuric chloride dose.

The significant increase in blood creatinine is an indication that the body's response to mercury exposure through mechanisms like the increased expression of metallothionein (metal-binding protein), production of glutathione, and activity of the antioxidant enzyme system (Zalups, 2000) was insufficient in managing the effects of 5 mg of $HgCl_2$ per kilogram body weight. Hence, the validation of the consideration that mercury is nephrotoxic.

In the current study, the nephrotoxic changes were associated with male rat subjects based on the study design. Therefore, the observations in the current study cannot be equally inferred to female rats. Also, according to Hazelhoff *et al.* (2012), renal impairment in female rats is lower compared to impairments in male rats though both show $HgCl_2$-induced renal injuries. The gender difference was described to be due to the expression of organic anion transporters (1 and 3) in the renal cortex which is higher in males than females and promoted by androgens while estrogen inhibits. Both of the organic anion transporters participate in anion/ alpha-ketoglutarate exchange at the proximal tubule, loop of Henle, and collecting ducts (Hazelhoff *et al.*, 2012).

In CG+$HgCl_2$-treated(D1) group (that is, $HgCl_2$ + 250 mg CG extract per kilogram body weight) and CG+$HgCl_2$-treated(D2) group ($HgCl_2$ + 500 mg CG extract per kilogram body weight), the desired expectation was a significant decrease in the

concentration of creatinine compared to the $HgCl_2$-treated group and a less evident or no change in creatinine concentration compared to the vehicle control group, respectively. Yet, that was not the case in this study. The blood creatinine was higher in the intervention groups compared to the $HgCl_2$-treated group, but the difference was insignificant. In comparison to the vehicle control group, the differences were significant compared to the intervention groups (Table 4). Ezeugwunne *et al*. (2017) using a different plant, showed similar findings pointing to kidney dysfunction. According to the results, there was a significant increase in serum creatinine and urea after *Sida corymbosa* leaf extract treatment. These observations point to kidney dysfunction where the interference with the normal function of the kidney leads to the elevation of the blood creatinine. Thus, the significant increase in this kidney function marker shows the toxic effects of $HgCl_2$ in combination with the extract. There is a dysfunctional excretory function of the nephron in the removal of toxic compounds. Histologically, degenerative structures were supporting the above-mentioned biochemical observations, indicating decreased kidney function. In the CG+$HgCl_2$-treated(D1) group compared to the $HgCl_2$-treated group, both groups exhibited dilation of the Bowman's capsule and tubular necrosis. The affected glomeruli were swollen with moderate damage, different from the extensive damage in the glomeruli and atrophy in the $HgCl_2$-treated group (figure 4 and 5).

Similar studies using extracts such as *Moringa oleifera* oil (Abarikwu *et al.*, 2017) and *Juglans sinensis* Dode extract (Beohm *et al*., 2002) have reported a decline in creatinine concentration in the intervention groups and a less evident difference compared to the $HgCl_2$-treated and the vehicle control group, respectively. Abarikwu *et al.* (2017) related the protection to the anti-peroxidative property of the *Moringa oleifera* oil to the kidney and Beohm *et al.* (2002) further associated kidney dysfunction

to the mitochondria of the renal proximal tubule cell which is a target of mercury toxicity.

Unlike the current study, while examining the protective effects of silymarin, Oda & El-Ashmawy (2012) noted an improvement in the intensity of the damage with necrotic and degenerative alterations localized at the corticomedullary junction and the renal cortex. Also, in a pretreatment case in rats, Alam (2007) showed protection against $HgCl_2$-induced injury. However, the tissues presented instances of congested glomeruli with reduced oedema. These results show that prophylaxis has the potential to protect against mercury-induced nephrotoxicity. However, this is contrary to the observations made in the current study. It may be an indication of the variation in the active compounds present in the extracts that influence their effectiveness in protecting the kidney. Also, the essential components, like flavonoids, important for their protective activity may be inadequate to produce the desired effects.

The higher creatinine levels in the treatment groups compared to the control groups (table 2) could depict a possible harmful effect of CG extract in combination with mercury on the kidney which is inconsistent with other similar studies focusing on nephroprotection against mercury-induced renal damage (Abarikwu *et al.,* 2017; Alam, 2007; Gado *et al.,* 2014). However, despite the aforementioned observations, the CG methanolic extract has been associated with nephroprotective activity in streptozotocin (STZ) - induced diabetic rats by Narsimhulu *et al*. (2019). Also, it is non-toxic up to concentrations of 2000 mg per kg body weight in healthy adult albino rats based on the OECD guidelines 423 (Kalpana *et al.,* 2018). It could be associated with the extract's component(s) that promote mercury bio-availability in circulation, such as vitamin C, whose content in CG ranges from 214.31 to 319.12 mg per 100 g dry weight (Jinazali *et al.,* 2017). It is an essential nutrient to the body known to maintain the flexibility of

blood vessels and improve blood circulation (Emmanuel *et al.*, 2016), and thereby, in the event mercury is bio-available in the blood, the improved circulation may increase the kidney's exposure to the toxin, hence the effects.

In the treatment groups, an increase in the concentration of the extract from 250 to 500 mg per kilogram of body weight tended to decrease the concentration of creatinine, though insignificantly (table 4). Considering the aforementioned possible harmful effects of CG extract, an increase in concentration could have produced more marked effects on the kidney, which was not the case. Another study with a similar study design focusing on *Eruca sativa* extract against mercury nephrotoxicity showed a dose-dependent potency in renal protective activity (Alam, 2007). Unlike the observation this current study, blood creatinine were reduced to a normal level, indicating the superior potency of *Eruca sativa* extract compared to CG extract.

CG extract's inability to significantly reduced the increased blood creatinine is supported by the degenerative changes in the glomeruli and tubules. The glomeruli showed extensive alterations while the tubular protection was minimal, and the Bowman's capsule was dilated with the glomeruli swollen. Also, tubular necrosis was evident (figure 5 and 6). Alam (2007) on the other hand, showed that a higher dose improved the tubules and glomeruli degenerative changes to within normal limits when using *Eruca sativa* seed extract. With a higher dose, the quantity and activity of the active compounds should increase, producing a more observable outcome (Kennedy & Wightman, 2011). However, in this study, the increase was not adequate to bring about a more desirable outcomes. This consideration is only relevant for CG extract concentrations of up to 500mg of extract/kg body weight.

Such variations between extracts of different plants may be a result of the difference in the content of active compounds like flavonoids and glycosides due to factors like

genetics, growth conditions, the timing of the harvest, and extraction techniques. The result of these variations is also true for plants of the same species since Narendhirakannan *et al*. (2007) showed the effectiveness of CG in adjuvant-induced arthritis, a disease state with a similar mechanism of action as mercury, that is, the generation of reactive oxygen species. The outcome, of the current study, is CG plant extract's inability to effectively influence mercury toxic effects. The aforementioned variations between the extracts can also be related to the management of mercury body burden. Since polyphenolic phytochemicals such as flavonoids can bind heavy metals (Hider *et al.*, 2001) like mercury due to the presence of hydroxyl groups, the body burden of mercury can be reduced (Magdalena & Brzóska, 2018), hence reducing tissue injury.

The concentration of blood urea in the $HgCl_2$-treated group was observed to be higher compared to the vehicle control (Table 2), a sign of nephrotoxicity in combination with the report on the creatinine concentration. Oda & El-Ashamawy (2012) made a similar observation, but the concentration of blood urea was lower (56.0 ± 0.58 mg/dL), showing the variability of urea concentration on exposure to $HgCl_2$. Similar to creatinine, it shows reduced functionality in the glomerular filtration process, leading to its accumulation in the blood. This observation is supported by extensive tubular and glomerular damage observed with features such as Bowman's capsule dilation, glomerular atrophy, and tubular necrosis (figure 4), compared to the vehicle control that had normal morphology (figure 3). Studies by Abarikwu *et al.* (2017) and Gado *et al.* (2014) also observed a disruption of normal kidney function in combination with changes observed in the concentrations of urea and creatinine due to the interference in their excretion by the kidney.

Concerning the histological findings, the interaction between the mercuric ion and the thiol functional group in proteins, which influences protein structures and inactivates thiol-containing enzymes, could play a major role in the toxic effects of mercury in the kidney tissues (Bernhoft, 2012; Spiller, 2018). Lack of functionality of the sulfhydryl-containing proteins, for example, Na+/K+ ATPase, alters the integrity of cells, interrupting the membrane potential and volume of organelles and the whole cell (Rana *et al*., 2018). Also, apoptosis has been linked to renal damage (Rana *et al*., 2018).

An increase in urea concentration is usually accompanied by an increase in creatinine concentration, as was the case in the current study. However, in a study by Abarikwu *et al*. (2017), a different observation was made where a rise in blood creatinine concentration was not accompanied by a noticeable increase in urea concentration in mercury-induced nephrotoxicity. Such an observation is also made in some medications, such as cimetidine and corticosteroids, which inhibit creatinine secretion into the urine at the proximal tubule and creatinine synthesis or release, respectively (Andreev *et al*., 1999). However, such a case is not a reflection of acute renal dysfunction or an influence on the glomerular filtration rate, which is a primary determinant of blood creatinine concentration (Andreev *et al*., 1999). In combination with the observational results of this study, the above-reported result presents a scenario where the nephrotoxicity of mercury brings about a variety of outcomes.

Instead of the CG extract influencing the reduction of the blood urea concentration in the treatment groups compared to the $HgCl_2$-treated group, there was no significant difference (table 6). This observation is in line with the degenerative changes in the glomeruli and tubules in the current study (figure 5 and 6). In similar studies using different plant extracts, there was a reduction in the blood urea concentration, showing

protection (Alam, 2007; Gado *et al.,* 2014). The significant increase is a feature of toxic plant extracts like the ethanolic root extract of *Jatropha gossypifolia* (Medubi *et al*., 2010) that are harmful to the kidney, leading to the retention of urea. However, CG methanolic extract is non-toxic at concentrations of 250 and 500 mg per kilogram body weight (Kalpana *et al*., 2018). As earlier mentioned, the increase may be a result of a component of the extract that facilitates the effects of mercury exposure. Also, according to Gonden *et al.* (2021), there is a possibility of the influence of non-renal influences such as diet, dehydration, upper gastrointestinal bleeding, and catabolic states in an instance of increased blood urea concentration. Generally, the extract was incapable of limiting the effects of mercury based on the level of blood urea.

Similar to the current study, pretreatment with vitamin E does not reverse increased blood creatinine and urea concentrations; instead, they were further enhanced (Agarwal *et al.*, 2010b). It points to mercury accumulation in kidney tissues due to mercury's influx, promoting tissue damage.

A decrease in the albumin concentration accompanied exposure to mercuric chloride, though insignificantly, in this study (table 8). Similar to the finding of the current study, Oda & El-Ashmawy (2012) also demonstrated a decrease in the blood albumin concentration, though the decrease in the $HgCl_2$-treated group was significant. An injury to the kidney is normally linked to a decrease in blood albumin concentration (ATSDR, 1999; Lang *et al*., 2018). However, the decline can be multi-factorial, reflecting a much broader variety of abnormalities (Lang *et al.*, 2018), such as the decline in protein synthesis (Samipillai *et al.*, 2013), increased epithelial degradation, and a systemic inflammatory response (Don & Kaysen, 2004; Haller, 2006). Considering the study by Oda & El-Ashmawy (2012), the change mainly points to a decline in protein synthesis.

Though lower serum albumin and kidney function can be related to various factors that are not clearly understood, in situations where the severity and prevalence of liver injury cannot be properly adjusted for, lower serum albumin and reduced kidney function are associated. In this case, it could signify a decline in kidney function as a result of inflammation and/or epithelial degradation. Additionally, albumin has been reported to be part of an adaptive response to oxidative stress in cells by controlling the expression of various genes linked to antioxidant enzymes. The process involves albumin's translocation into cells (Weber *et al*., 2004). In combination with decreased synthesis, such factors can explain its decrease.

Unlike other blood biochemical parameters, albumin concentrations were lower in the *Cleome gynandra* extract-treated groups compared to both controls (table 2). Only the group receiving the highest concentration of CG extract, that is, 500 mg/kg body weight was significantly different (p = 0.002) when compared to the vehicle control group (table 8). Similar to the finding of this current study, Oda & El-Ashamawy (2012) also demonstrated an insignificant decrease in the blood albumin concentration in the treatment group. The decreased blood albumin seen in CG-treated groups are difficult to explain considering the data collected. However, it indicates a hypoalbuminemic property and the extract's inability to maintain albumin levels within a normal range (3.1 - 4.6 g/dL) on exposure to 5 mg of $HgCl_2$ per kg body weight. In other studies, blood albumin has been found to increase with exposure to mercury (Abarikwu *et al*., 2017; Lukačínová *et al*., 2011). It could be linked to the mercury's ionic species' propensity to bind –SH group in albumin for transportation purposes, thereby, increasing albumin production (ATSDR, 1999; Chunmei *et al.*, 2014; Nordberg *et al*., 2005).

5.2 Histological changes associated with the effects of *Cleome gynandra* (CG) against a low concentration of mercuric chloride

At the low concentration of mercuric chloride (0.5 mg per kg body weight), at the end of the experiment, marked histological changes were absent in the CG extract-treated group compared to the $HgCl_2$-treated group. From the histological examinations in this study, it could also be seen that the degree of renal injury in the $HgCl_2$-treated group increased with the progression of the days, from 12.5-25% morphological changes on day 2 to approximately 100% changes on day 8 (figure 7, 8, 9, 10, 11, and 12). This finding could be a sign of mercury's retention in the body and its prolonged half-life in the body of about one to two months mostly attributed to its strong interaction with selenium and -SH groups (ATSDR, 1999; Nordberg *et al*., 2005) - an observation also made by Park & Zheng (2012). As time progresses after and with periodic exposure, the more the proportion of mercury in the body is found in the kidney. The excretion process depends on the dose of mercury and occurs in two phases, with the initial phase being rapidly followed by a slow second phase of excretion. Though mercury is excreted through urine, faecal matter, breath, sweat, and saliva, the rate of excretion is not adequate to limit mercury's toxic effects on the body even at such a low concentration (Park & Zheng, 2012). With an increase in mercury concentration in the tissue, the more pronounced the alterations become.

In the current study, the morphology of the glomeruli showed degenerative features with inflammation and the Bowman's capsule exhibited dilation in the $HgCl_2$-treated group. Unlike our findings, Nicholson *et al*. (1985) determined that for mercury at a low concentration of 0.5 mg per kg body, the structural damage in the kidney is limited to the pars recta where primarily mitochondria metabolism is affected through the inhibition of certain tricarboxylic acid enzymes as was indicated by the decreased

metabolism of succinate and alpha-ketoglutarate by the tubular cells of the kidney. Gökc *et al*. (2016) on the other hand showed additional degeneration such as tubular dilation and glomerular lobulation in a sub-acute study, acknowledging that the alterations are due to mercury-induced necrosis and apoptosis. These observations could cumulatively offer a perspective on the degenerative effect of exposure to low mercury concentrations. A probable justification for these effects is mercuric ion affinity for sulfhydryl and selenohydryl functional groups, altering proteins' structures and inhibiting their functions (Bernhoft, 2012). Accompanied by features such as mitochondrial dysfunction and lipid peroxidation that alter membrane integrity, therefore, presence of mercuric ions leads to kidney degeneration (Gaschler & Stockwell, 2017). Often, the majority of the mercuric ions are detected in the outer stripe of the outer medulla and the cortex. This is a reasonable finding since the proximal tubule and the glomerulus span the two renal zones (Oda & El-Ashmawy, 2012).

In the treatment group, the alterations were maintained at a minimal level (below 12.5% severity) throughout the experimental period (figure 8, 10, and 12). While using luteolin (a flavonoid), Tan et al. (2018) showed nephroprotection. In the study, the protection was associated with reduced mercury-induced oxidative stress and inflammatory response due to the increased glutathione concentration, reduced malondialdehyde formation, and activation of reduced nuclear factor kappa B (NF-κB). Also, cell death was curbed. These observations may point to the hypothesis of the CG extract has a similar mechanism of action.

The objectives of this study were to determine the protection of CG (*Cleome gynandra*) extract against mercury-induced renal damage at both a probable lethal and low doses of mercury chloride. Results from the study showed that there was no significant

protection against mercury nephrotoxicity (5 mg $HgCl_2$/kg bwt) based on the findings on the blood biochemical parameters of kidney function and the histological changes, but at low, non-lethal level of mercury (0.5 mg $HgCl_2$/kg bwt), the CG extract offered protection to the kidney based on the semi-quantitative analysis.

CHAPTER SIX: CONCLUSIONS

6.1 Conclusions

In conclusion, at a low mercury concentration (0.5 mg/kg body weight), *Cleome gynandra* (CG) was found to be protective against the toxic effects of a low concentration of mercury (0.5mg/kg body weight) based on the histological findings. On the other hand, the CG extract was ineffective against nephrotoxicity brought about by a high dose of mercury (5mg/kg body weight). Therefore, it does not pose as a potent nephroprotective agent due to its inability to maintain kidney function at a normal range based on observations made on the biochemical parameters and histological changes.

This study shows the importance of *in vivo* experiments. Also, it may be inadvisable to use CG as a protective nutritional product in heavy metal endemic areas where the frequency of exposure to high concentrations is high.

6.2 Recommendations

In light of the findings of this study, it is recommended that:

1) The results of this study serve as an additional information resource on the potential of *Cleome gynandra* in management of various conditions and an advancement to the knowledge about the *C. gynandra*.
2) The pre-treatment with methanolic extract of *C. gynandra* is inadvisable in the management of high mercury concentrations in experimental rat models.
3) Post-treatment with CG extract can be investigated to determine the extract's effectiveness against mercury-induced renal damage.
4) Further studies can be considered in expanding the search for particular extracts from other plant species that are more effective, while at the

same time identifying mercury dose levels that the extracts can offer protection against.

5) 10% dimethyl sulphoxide is a suitable solvent for plant extracts in experimental animal study designs for acute conditions while using rats as a model for disease condition.

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APPENDICES

Appendix 1: Research approval

OFFICE OF THE DIRECTOR OF GRADUATE STUDIES AND RESEARCH
UNIVERSITY OF EASTERN AFRICA, BARATON
P.O. BOX 2500-30100, Eldoret, Kenya, East Africa

B04060520201 May 6, 2021

TO: Odhiambo Jackson Ochieng
Dept. of Medical Biochemistry, School of Medicine
Moi University

Dear Jackson,

RE: The Protective Potential Cleome gynandra Leaf Etract against Mercury Chloride Mediated Kidney Damage in Male Wistar Albino Rats

This is to inform you that the Research Ethics Committee (REC) of the University of Eastern Africa Baraton has reviewed and approved your above research proposal. Your application approval number is UEAB/REC/04/05/2021. The approval period is 6th May, 2021 – 6th May, 2022.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by the Research Ethics Committee (REC) of the University of Eastern Africa Baraton.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to the Research Ethics Committee (REC) of the University of Eastern Africa Baraton within 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to the Research Ethics Committee (REC) of the University of Eastern Africa Baraton within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to the Research Ethics Committee (REC) of the University of Eastern Africa Baraton.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Sincerely yours,

Prof. Jackie K. Obey, PhD
Chairperson, Research Ethics Committee

University of Eastern Africa, Baraton
06 MAY 2021
P.O. BOX 2500-30100 ELDORET
Research Ethics Committee

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Appendix 2: Raw data for the biochemical parameters

Table 9: Measured levels of creatinine concentration in mg/dL for the experimental animals in the 4 groups.

Animals	Vehicle Control	$HgCl_2$-treated	CG+$HgCl_2$-treated(D1)	CG+$HgCl_2$-treated(D2)
1.	0.272	3.602	2.917	3.790
2.	0.274	0.343	3.158	2.471
3.	0.318	1.732	2.738	3.537
4.	0.299	2.554	3.406	3.437
5.	0.307	1.302	3.381	1.229
Mean±SD	0.294±0.020	1.907±1.238	3.120±0.291	2.893±1.056

Table 10: Measured levels of blood urea concentration in mg/dL for the experimental animals in the 4 groups.

Animals	Vehicle Control	$HgCl_2$-treated group	CG+$HgCl_2$-treated(D1) group	CG+$HgCl_2$-treated(D2) group
1.	19.154	136.824	127.975	129.012
2.	12.770	18.090	149.426	111.453
3.	11.789	69.728	117.558	98.152
4.	18.174	120.302	118.678	103.893
5.	13.974	47.466	138.533	43.237
Mean±SD	15.172±3.299	78.482±49.595	130.434±13.568	97.149±32.299

Table 11: Measured levels of blood albumin concentration in g/dL for the experimental animals in the 4 groups.

Animals	Vehicle Control	$HgCl_2$-treated group	CG+$HgCl_2$-treated(D1) group	CG+$HgCl_2$-treated(D2) group
1.	4.499	3.424	3.094	0.605
2.	3.441	3.545	2.919	3.225
3.	3.832	3.336	3.288	0.716
4.	3.733	3.046	2.445	2.958
5.	4.252	3.655	3.307	3.083
Means±SD	3.951±0.422	3.401±0.232	3.011±0.354	2.117±1.334

Appendix 3: Phytochemicals and minerals present in *Cleome gynandra*

Table 12: Phytochemicals and minerals of *Cleome gynandra* represented in published articles

Phytochemicals	**Examples**
Vitamins	- Vitamin C (Gowele *et al.*, 2019) - Vitamin A (Gowele *et al.*, 2019) - Provitamin A carotenoids (Gowele *et al.*, 2019) including beta-carotene, alpha-carotene, and beta-cryptoxanthin (Moyo & Aremu, 2021) - Vitamin E including alpha-tocopherol, beta-tocopherol, and gamma-tocopherol (Moyo & Aremu, 2021).
Other carotenoids	- Lutein (Moyo & Aremu, 2021) - Violaxanthin (Moyo & Aremu, 2021)
Flavonoids	- Glycosides of quercetin, kaempferol, and isohamnetin (Lingegowda *et al.*, 2012; Omondi *et al.*, 2017)
Triterpene glycosides	- Saponins (Moyo *et al.*, 2013)
Others	- Iridoids, gallotannins, free gallic acid (Moyo *et al.*, 2013), alkaloids, anthraquinones, cardiac glycosides, phenols, sugars, triterpenes (Adhikari & Paul, 2018), steroids, and tannins (Deepashree & Gopal, 2014).

Minerals	Examples
Macronutrients	- Sulfur, potassium, phosphorous, magnesium, and calcium (Moyo & Aremu, 2021; Omondi *et al.*, 2017).
Micronutrients	- Manganese, zinc, and iron (Moyo & Aremu, 2021; Omondi *et al.*, 2017)

Phyto Chemicals	Test name	HE	CE	AE	ME
Flavonoids	Sodium hydroxide test	-	+	++	++
Alkaloids	Dragendorff's test	-	++	+	+
Steroids	Salkowski's test	+	+	-	+
Terpenoids		+	-	-	-
Glycosides		-	-	-	+
Tannins	Ferric chloride test	-	+	++	+
Saponins	Foam test	-	-	+	-

Figure 13: Phytochemical screening of *Cleome gynandra* (DeepaShree & Gopal, 2014)