بسم الله الرحمن الرحيم

Sudan University of Science and Technology
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Antioxidant and Nephroprotective Effects of *Cassia occidentalis* Leaves Methanolic Extract in Rats

التأثيرات المضادة للأكسدة والواقية للكلى للمستخلص الميثيلى لأوراق السوريب في الجرذان

A Thesis Submitted in Fulfillment of the Requirements of Master Degree in Pharmacology

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الآيـة

قال تعالى:

﴿هُوَ ٱلَّذِيٓ أَنزَلَ مِنَ ٱلسَّمَآءِ مَآءٗ لَّكُم مِّنۡهُ شَرَابٞ وَمِنۡهُ شَجَرٞ فِيهِ تُسِيمُونَ﴾

صدق الله العظيم

الآية (١٠) من سورة

النحل

Dedication

I dedicate this work to

my parents, brothers, friends and my teachers.

Mujtaba

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First of all, thanks and praise to Almighty Allah, for giving me health, faith and strength to accomplish this work.

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List of Abbreviations

COLM: *Cassia occidentalis* Leaves Methanolic Extract

ALP: Alkaline Phosphatase

AST: Aspartate Transaminase

ALT: Alanine Transaminase

DEMSO: Dimethyl Sulfoxide

DPPH: 1,1- Diphenyl-2- Picryl-hydrazyl

RNS: Reactive Nitrogen Species

ROS: Reactive Oxygen Species

ANOVA: Analysis of Variance

SPSS: Statistical Package for Social Sciences

SOD: Superoxide Dismutase

Abstract

Cassia occidentalis (Sorib) is a rich source of bioactive compounds with a variety of pharmacological therapeutic properties and considerable effectiveness against oxidative stress-induced severe renal disease. In this study *C. occidentalis* leaves methanolic extract (COLM) was evaluated for its antioxidant and nephroprotective activities. The phytoconstituents of the leaves extract was also investigated. The antioxidant activity of the extract was measured using 1,1- diphenyl-2- picryl-hydrazyl (DPPH) radical scavenging assay.

Nephroprotective activity of COLM was assessed using Wistar albino rats. Seventy rats were divided randomly into seven groups of ten rats each; control group, nephrotoxic group, rats were injected with gentamicin only at a dose of 80mg/kg, intraperitoneally (IP) for 8 days. Standard drug group, animals were injected with gentamicin (80 mg/kg IP), and at the same time they received standard drug silymarin at a dose of 100mg/kg orally for 8 days. Low and high doses of the plant groups, rats were injected with gentamicin (80mg/kg IP), with concurrent administration of *C. occidentalis* leaves methanolic extract at a dose of 200 or 400 mg/kg orally for 8 days. Toxicity groups, rats were given *C. occidentalis* leaves extract only at a dose of 200 or 400 mg/kg orally for 8 days. The levels of urea, creatinine, uric acid, and total protein in the blood were used to assess kidney function. Blood constituents, and relative kidney weight were also measured. Phytoconstituents of COLM comprise tannins, saponins, coumarins, and alkaloid as active components and devoid of flavonoids, and sterols. COLM exhibited moderate antioxidant activity (50 ± 0.04%) compared to the standard antioxidant agent propyl gallate (93 ± 0.01%) using DPPH radical scavenging assay.

Administration of COLM at a dose of 200 and 400 mg/kg for 8 days significantly ($P < 0.05$) lowered the levels of urea compared to the gentamicin group. The levels of creatinine, uric acid and total protein were insignificantly changed ($P > 0.05$) at day 8 in treated groups compared with control. COLM showed no significant changes compared to the standard drug Silymarin. Relative weight of kidneys was significantly increased in treated groups, except toxicity groups, compared to that of control. The results concluded that the methanolic extract of *C. occidentalis* leaves might possess nephroprotective activity in rats. This effect may be due to antioxidant activity or the chemical constituents of the plant.

Arabic Abstract

المستخلص

يعد *C. occidentalis* (السوريب) مصدرا غنيا بالمركبات النشطة بيولوجيًا مع خصائص دوائية علاجية وفعالية كبيرة ضد أمراض الكلى الحادة التي يسببها الإجهاد التأكسدي.

في هذه الدراسة قيم المستخلص الميثانولي لأوراق*C. occidentalis* لأنشطته المضادة للأكسدة والواقية للكلى. كما تم ايضا التقصى عن المكونات النباتية لمستخلص الأوراق. تم قياس النشاط المضاد للأكسدة للمستخلص باستخدام فحص الكسح الجذري 1,1- diphenyl-2- picryl-hydrazyl (DPPH).قيم النشاط الكلوي لـCOLM باستخدام الجرذان. تم تقسيم سبعين جرذا عشوائيا إلى سبع مجموعات كل منها تحتوى على عشرة فئران، مجموعة تحكم، مجموعة تحكم للتسمم الكلوي تم فيها حقن الفئران بالجنتاميسين فقط بجرعة 80 مجم / كجم داخل الصفاق (IP) لمدة 8 أيام. مجموعة الدواء القياسى تم حقن الجرذان بالجنتامايسين (80مجم / كجم IP) وفي نفس الوقت اعطي عقار سيليمارين القياسي بجرعة 100 مجم / كجم عن طريق الفم لمدة 8 أيام.

فى الجرعات المنخفضة والعالية فى مجموعتى النبات ؛ تم حقن الجرذان بالجنتاميسين (80مجم / كجمIP) مع الإعطاء المتزامن لمستخلص أوراق النبات الميثانولي بجرعة 200 أو 400 مجم / كجم عن طريق الفم لمدة 8 أيام. مجموعتى السمية، أعطيت الفئران مستخلص أوراق *C. occidentalis* فقط بجرعة 200 أو 400 مجم / كجم عن طريق الفم لمدة 8 أيام. تم قياس مستويات اليوريا والكرياتينين وحمض البوليك والبروتين الكلي في الدم لتقييم وظائف الكلى .كما تم قياس مكونات الدم والوزن النسبي للكلية. تشتمل المكونات النباتية لـ COLM على التانينات ، والصابونين ، والكومارين ، والقلويدات كمكونات نشطة، كما انها خالية من مركبات الفلافونويد والستيرولات. أظهر COLM نشاطًا معتدلًا مضادًا للأكسدة (50 ± 0.04 ٪) مقارنة بعامل قياسى مضاد للأكسدة propyl gallate (93 ± 01.0٪) باستخدام مقياس الكسح الجذرى (DPPH).

أظهر إعطاء COLM بجرعة 200 و 400 مجم / كجم لمدة 8 أيام انخفاضًا ملحوظًا في مستويات اليوريا مقارنة بمجموعة الجنتاميسين. اظهرت مستويات الكرياتينين وحمض البوليك والبروتين الكلي تغيرات غير معنوية (P> 0.05) في اليوم الثامن في المجموعات المعالجة مقارنة بالمجموعة الضابطة. لم يظهر COLM فروقًا ذات دلالة إحصائية عن تلك التي لوحظت بواسطة عقار سيليمارين القياسي. زاد الوزن النسبي للكلى معنويا في المجموعة المعالجة ما عدا مجموعة السمية مقارنة مع

مجموعة التحكم. خلصت النتائج إلى أن المستخلص الميثانولي لأوراق *C. occidentalis* قد يكون له نشاط كلوى في الفئران. قد يكون هذا التأثير بسبب نشاط مضادات الأكسدة أو المكونات الكيميائية للنبات.

INTRODUCTION

Medicinal plants have been utilized as traditional therapies for several human and animal ailments for thousands of years. Plants have a variety of active substances known as phytochemicals that produces well-defined physiological activity on the human body and animal. These phytochemicals comprise a variety of compounds such as alkaloids, tannins, glycoside, saponins, flavonoids and terpenes (Sadiq *et al.*, 2012). Because some chemical substances such as drugs and toxins can produce negative effects in many organs, including the kidneys; using herbal plant extracts for nephroprotection is the ideal way in traditional medicine to cure kidney damage without causing any negative side effects (Gaikwad *et al.*, 2012).

The kidney is the principal organ in the urinary system that eliminates waste materials from the blood and excretes them in urine. It plays an important role in maintaining electrolytes balance, fluid homeostasis and blood pressure and regulating acid base balance and blood calcium level, and is involved in the process of gluconeogenesis (Ramesh *et al.*, 2014 and Bindu *et al.*,2016). Acute and chronic renal failure, uremia, and anemia are all disorders that damage the kidneys (Ramesh *et al.*, 2014). Nephrotoxicity is a common condition that occurs when the body is exposed to certain medications or chemicals such as aminoglycosides. Therefore, prolong use of aminoglycosides e.g. gentamicin is complicated by a great risk of nephrotoxicity (Reddy *et al.*, 2016 and Hafez *et al.*, 2019).

In folkloric medicine, medicinal plants are assumed to be the best treatment to cure nephrotoxicity (Adeneye and Benebo, 2008). Recently, there is an extraordinary increase in the herbal medicine research as an alternative source of remedies to treat various diseases (Ahmed *et al.*, 2019). In traditional medicine in Sudan, several plant have been used successfully to cure a variety of illnesses including renal disorders such as Gum Arabic, *Boscia sengelensis*, and goriander, celery and caraway (Ali *et al*., 2020).

Hence the present study was conducted due to the limited success associated with the use of conventional drugs that are used to treat renal disorders as well as the significant adverse effects associated with the use of these drugs.

Objectives of the study

General objective

1. To evaluate the antioxidant and nephroprotective effects of the methanolic extract of *Cassia occidentalis* leaves against gentamycin induced nephrotoxicity in Wistar rats.

Specific objectives

1. To evaluate the antioxidant activity of *Cassia occidentalis* leaves using DPPH radical scavenging assay.
2. To identify the chemical constituents of *Cassia occidentalis* leaves.
3. To assess the changes in the blood biochemical constituents in rats treated with *Cassia occidentalis* extract and gentamycin.
4. To evaluate the haematological changes in rats treated with *Cassia occidentalis* extract and gentamycin.
5. To determine the pathological changes in kidney produced by *Cassia occidentalis* and gentamycin.

CHAPTER ONE
1. Literature Review

1.1 Importance and function of the Kidney

The kidney is the principal organ in the urinary system that eliminates waste materials from the blood and excretes them in urine. It plays an important role in maintaining electrolyte balance, fluid homeostasis and blood pressure and regulating acid base balance and blood calcium level, and is involved in the process of gluconeogenesis (Bindu *et al.*, 2016 and Ramesh *et al.*, 2014). They guard blood volumes, filter the blood to form urine regulate water, electrolytes, acid/base balance, produce some hormones and participate in metabolism of others at rest an estimated 20% of cardiac output flows through the kidney where they are filtered and reconditioned (Mahmoud *et al.*, 2015).

1.2 Kidneys ailments

Kidneys are one of the vital organs affected by accumulation of toxic substances in the body, exposure to toxic substances can cause injury or death of tissues in the kidney resulting in leakage of essential biomolecules into the blood stream alongside with histo-morphological changes (Isah *et al.*, 2018). There are many diseases affecting the kidney such as acute and chronic renal failure, uremia and anemia (Ramesh *et al.*, 2014). Nephrotoxicity is a major side effect of many drugs like some NSAIDs, aminoglycoside antibiotics, etc (Tiwari*et al.*, 2016). Renal failure occurs when the excretory function of the kidney fails to filter out metabolic waste products such as creatinine and blood urea nitrogen. Some of the important nephrotoxic agents include heavy metals, antineoplastic and antimicrobial agents (Chandavarkar *et al.*, 2017). Furthermore, kidneys are highly

vascularized, compound tubular glands, organized principally from numerous, closely packed and uriniferous tubules. Also it produces or activate hormones that are involved in calcium metabolism, erythrogenesis and regulation of blood pressure (Hafez *et al.*, 2019). Recognition of drug-induced nephrotoxicity as a significant contributor to kidney diseases including acute kidney injury, and chronic kidney disease, has gained increasing momentum in recent times. Nephrotoxicity constitutes a whole gamut of disorders reflecting damage to different nephron segments as a consequence of individual drug mechanisms (Aiswarya *et al.*, 2018).

Renal failure occurs when the kidney's excretory function fails to filter metabolic waste products like creatinine and blood urea nitrogen out of the body. The most common nephrotoxic substances include heavy metals, antineoplastics, and antibiotics (Chandavarkar *et al.*, 2017). Acute renal failure is a major side effect of aminoglycosides and accounts for 10–20% of patients using this medicine and percent of cases can be directly attributed to drugs such as antibiotics because the major factor implicated in the acute kidney injury due to indigenous functions of kidney to excrete them. This acute renal injury often leads to renal failure which in turn is associated with other pathological manifestations such as sepsis, cardiovascular disorders and diabetes (Srivastava *et al.*, 2018 and Khan *et al.*, 2015). Kidney damage caused by gentamicin shows the same conditions with renal function impairment is clinically characterized by elevated blood urea nitrogen and creatinine plasma levels, albuminuria, reduced glomerular filtration rate renal dysfunction (Saputri *et al.*, 2017).

1.3 Gentamicin-induced nephrotoxicity

Gentamicin-induced renal damage is a widely used model for inducing nephrotoxicity in experimental animals (Abhirama *et al.*, 2018). Gentamicin is an aminoglycoside isolated from *Micromonospora purpurea*. It has a hexose ring to which various amino sugars are attached by

glycosidic linkages (Khan *et al.,* 2015). It is widely used in clinical practice for the treatment of Gram-negative infections. Its usage is complicated by the high risk of nephrotoxicity and ototoxicity, especially when used over long periods of time (Hafez *et al.,* 2019 and Reddy *et al.,* 2016). However, it widely used due to its relatively low cost and rapid bactericidal action (Heidarian *et al.,* 2017).

In its elimination route, gentamicin accumulates in the renal proximal tubular cells through the megalin/cubilin complex receptor, which is responsible for transportation inside the cell. Aminoglycoside-induced nephrotoxicity is characterized by slow rises in serum creatinine, tubular necrosis and marked decreases in glomerular filtration rate and in the ultrafiltration coefficient. Other effects include inhibition of protein synthesis and modulation of gene expression, mitochondrial alterations, or inhibition of enzymes located on the cytosolic side of the pericellular membrane, or involved in the uptake and intracellular distribution of the drug to the corresponding targets. Activation of the renin-angiotensin system and the ensuing local vasoconstriction appear to be primarily responsible for the decrease in the glomerular filtration, with severe necrosis of renal proximal convoluted tubules followed by failure of renal functions (Salgueiro and Nunez, 2016; Khan *et al.,* 2015; Rodrigues *et al.,* 2014; Gaikwad *et al.,* 2012 and Martínez-Salgado *et al.,* 2007). Pathological findings can be seen as proximal tubular edema, tubular necrosis, and desquamation, as well as inflammation and diffuse interstitial edema (Rodrigues *et al.,* 2014).

The damage is proposed to occur through pathological mechanisms such as apoptosis, necrosis, production of oxidative stress and by augmenting the levels of endothelin I and monocyte/macrophages infiltration, gentamicin is thought to augment the production of reactive oxygen species, such as hydrogen peroxide, super oxide anions, hydroxyl

radicals and reactive nitrogen species in the kidney (Srinivasan *et al.,* 2015). Gentamicin also acts as an iron chelator and the iron-gentamicin complex is a potent catalyst of radical (Chatterjee *et al.,* 2012). The cellular antioxidant status plays an important role in determining the susceptibility to oxidative damage which might alter in response to oxidative stress (Aiswarya *et al.*,2018).

1.4 Standard drug (Silymarin)

Silymarin is a herbal medicines extracted from the dried seeds of *Silybum marianum*. The plant contains four flavonolignan isomeric components (silybin, isosilybin, silychristin, and silydianin), and Silybin. Silymarin is taken orally and is mainly excreted through bile as conjugates. Silymarin has numerous clinical uses in the treatment of several diseases such as liver disorders, radiation toxicity and viral hepatitis as a result of its anti-oxidative, anti-lipid peroxidative, antifibrotic, anti-inflammatory, immunomodulating, promotion of protein synthesis and liver regenerating effects (Rajaratnam *et al.*, 2014 and Elagib *et al*., 2014 and Ahmed *et al.,* 2019).

1.5 Oxidative stress and antioxidant plants

Oxidative stress plays a vital role in the pro-inflammation, and apoptosis induced nephrotoxicity (Edeogu *et al.*, 2020). Nephrotoxic drugs generate hydrogen peroxide in renal cortex mitochondria and can also enhance the generation of reactive oxygen species (ROS). Abnormal production of ROS may damage some macromolecules to induce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage. The alteration in kidney functions induced by lipid peroxidation is a proximal event in the injury cascade of gentamicin mediated nephrotoxicity (Chatterjee *et al.,* 2012).

It is known that antioxidant rich herbs possess significant activity against various diseases condition characterized by induced oxidative stress. The cellular antioxidant mechanism plays an important role in determining the susceptibility to oxidative damage which might alter in response to oxidative stress. Several studies have claimed antioxidant property of drugs as crucial for their nephroprotective effects in gentamicin induced renal damage (Aiswarya *et al.,* 2018).

1.6 Nephroprotective plants

Nephroprotective agents are the substances which possess protective activity against nephrotoxicity. Ancient literature has prescribed various herbs for the cure of kidney diseases (Gaikwad *et al.,* 2012).

A number of herbs are traditionally used worldwide for alleviation of drug or toxin induced hepatic and renal disorders (Naggayi *et al.,* 2015). Several plants have been reported to possess potential nephroprotective activity, such as methanol and aqueous extracts of *Biophytum sensitivum* (Chandavarkar *et al.,* 2017), the aqueous leaf extract of *Aloe barbadensis* (Chatterjee *et al.,* 2012), *Carica papaya* seed extract (Naggayi *et al.,* 2015), Aqueous Extract of *Pimpinella anisum* (Aiswarya *et al.,* 2018). Gum Arabic (Tahir *et al.,* 2016), whole plant ethanol extract of *Biophytum sensitivum* (Abhirama *et al.,* 2018). These plants were evaluated as nephroprotective agents in experimental animals using different experimental models such as gentamicin, paracetamol and cisplatin to induced nephrototoxicity.

1.7 Phytoconstituents as nephroprotective agents

Medicinal plants have curative properties due to the presence of various phytocontituents. Traditionally, numerous herbs are prescribed for the cure of renal disorders. Co-administration of several medicinal plants possessing the nephroprotective activity along with different nephrotoxic agents which may attenuate its toxicity (Singh *et al.,* 2016). Various parts

of the medicinal plants contain major chemical constituents such as quercetin, quercetin-3-glucoside, isorhamnetin-4-glucoside, xylose, galactose, glucose, mannose, organosulfur compounds, allyl sulfides, flavonoids, flavenols, cyclically, selenium, thiosulfinates, and sulfur and seleno compounds (Chinnala *et al.*,2017). Plants contain a wide variety of free radical scavenging molecules, such as flavonoids, anthocyanins, carotenoids, dietary glutathione, vitamins and endogenous metabolites that are rich in antioxidant activities, phenolic compounds, including phenolic acid and flavonoids have been recognized as having health-related properties, including anticancer, antiviral and anti-inflammatory activities (Kathirvel and Sujatha, 2012).

1.8 Plant used in this Study

1.8.1 Classification of *Cassia occidentalis*

According to Manikandaselvi *et al.*, (2016) *C. occidentalis* classified as follow:

Kingdom: Plantae
Class: Dicotyledonae
Order: Fabales
Family: Fabaceae
Sub-family: Caesalpinioideae
Genus: Cassia - Senna
Species: *Cassia occidentalis* (Fig.1)

1.8.2 Description

Senna is the common name of 13 species of the genus, subtribe Cassiinae, tribe Cassieae, subfamily Caesalpinioideae (Leguminosae), family Fabaceae. The genus Senna, considered paraphyletic, derives from the subdivision of the complex genus **(**Ferreira *et al.*, 2010).

Fig.1. *Cassia occidentalis* leaves, (photo taken by Mujtaba, A. Abolgassim, Nyala-South Darfur area).

C. occidentalis belongs to the Family Fabaceae and locally known as "Sorib" (Mendes and Carlini, 2007). It is an erect, somewhat branched, smooth, semi-woody, fetid herb or shrub, 0.8–1.5 m tall, taproot, hard, stout, with a few lateral roots on mid-section. This plant species varies from a semi-woody annual herb in warm temperate areas to a woody annual shrub or sometimes a short-lived perennial shrub in frost free areas. The stem of the plant is reddish purple. The young ones are 4-sided, becoming rounded with age (Yadav *et al.,* 2010). Leaves are alternate, even pinnately compound, each one with 4–6 pairs of nearly sessile, opposite leaflets, with a fetid smell when crushed, each leaflet 4–6 cm long, 1.5–2.5 cm wide, ovate or oblong, lanceolate with a pointed tip and fine white hairs on the margin (Singh *et al.,* 2016). The rachis has a large, ovoid, shining, dark purple gland at the base. Stipules are 5–10 mm long, often leaving an oblique scar. Inflorescence is a compound of axillary and terminal racemes. The flower is perfect, 2 cm long with 5 yellowish green sepals with distinct red veins and 5 yellow petals. The fruit is a dry, dehiscent, transversely partitioned, faintly recurved, laterally compressed, sickle shaped legume (pod), 7–12 cm long, 8–10 mm wide, with rounded tip and containing 25–50 seeds. Seeds are oval shaped, 3.5–4.5 mm wide, flattened; pale to dark brown, slightly shiny, smooth and with a round pointed tip (Singh *et al.,* 2016; Nassar *et al.*, 2011 and Yadav *et al.*, 2010).

1.8.3 Local Names

In Sudan, *C. occidentalis* locally known as "Sorib" (Mendes and Carlini, 2007). In Nigeria, this plant is locally called Sanga-sanga or Rai dore in Hausa language Akidi agbara in Igbo language and Abo rere in Yoruba language (Isah *et al.,* 2018). It is commonly known as coffee senna, is native to tropical South America and found in almost entire Brazilian territory (Nassar *et al.,* 2013 and Ferreira *et al.,* 2010). It’s also

called Coffee senna, fetid cassia, and Negro Coffee (English). In India it is known by its various vernacular names, the most commonly used ones are Kasamarda, Kaasaari (Ayurveda), Kasaundi, Bari Kasaundi (Hindi), Kasondi (Unani), Doddaagace (Kanad), Ponnaviram, Ponnarviriam (Malyalam), Kasinda (Telgu), Paeyaavarai and Thagarai (Yadav *et al.*, 2010). It is called as Kasmard in Sanskrit, Kasondi in Hindi and Coffee Senna in English (Gautam and Navneet, 2014) see table 1.

1.8.4 Distribution

C. occidentalis is distributed throughout the tropics and subtropics including United States from Texas to Iowa eastward, Asia, India, Australia, and Africa under open condition. In India, it is a common weed found throughout India (up to an altitude of 1500 m), from Jammu and Kashmir to Kanyakumari and used for a variety of purposes in indigenous and folk medicines. In Haryana, it grows widely immediately after the rain and started disappearing in the beginning of cold weather (Ferreira *et al.*, 2010; Yadav *et al.*, 2010 and Odeja *et al.*, 2017). In Sudan, it is found in south Darfur and Kurdufan area (Mendes and Carlini, 2007).

1.8.5 Chemical constituents

The chemical constituents isolated from *C. occidentalis* leaves are alkaloids, flavonoids, tannins, saponins, sterols, triterpenes, quinines phlobatannins, chrysophanol, emodin, physcion, tetrahydroanthracene, derivative, germichrysone, sennoside, anthraquinone, fatty oils, glycosides, galactomannan and polysaccharides (Mendes and Carlini, 2007; Kathirvel and Sujatha, 2012 and Verma *et al.*, 2010). It also contains achrosin, aloe-emodin, emodin, anthraquinones, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chryso-obtusin, chrysophanic acid, chrysarobin, chrysophanol chrysoeriol, emodin, essential oils, funiculosin, galactopyranosyl, helminthosporine islandicine, kaempferol, lignoceric acid,

Table (1). Local names of *Cassia occidentalis*

Country	Local Name	References
Sudan	Sorib	(Mendes and Carlini, 2007).
Nigeria	Sanga-sanga	(Isah *et al.* 2018)
South America	coff ee senna	(Ferreira *et al.*, 2010)
India	Bari Kasaundi	(Yadav *et al.*, 2010)
Sanskrit	Kasmard	(Gautam and Navneet , 2014).

linoleic acid, linolenic acid, mannitol, mannopyranosyl, matteucinol, obtusifolin, obtusin, oleic acid, physcion, quercetin, rhamnosides, rhein, rubrofusarin, sitosterols, tannins, and xanthorine (Yadav *et al.,* 2010).

1.8.6 Traditional uses

In traditional medicine, various parts of *C. occidentalis* (seeds, roots, leaves and stems) have been widely used as a laxative, analgesic, febrifuge, diuretic, hepatoprotective, vermifuge and colagogo as well as for the treatment of tuberculosis, gonorrhea, dysmenorrhea, anemia, cough, convulsion, throat inflammation, fever, asthma, malria, filariasis, flu, skin, liver and urinary tract disorders (Manikandaselvi *et al.,* 2016 and Silva. *et al.,* 2011). Young leaves boiled with water are used for treating kidney dysfunction (Saputri *et al.,* 2017). The leaves are also used as paste on healing wounds, sores, itch, cutaneous diseases, bone fracture, fever, ringworm, treatment of yaws, scabies, skin diseases and throat infection. The leaves of this plant are chiefly utilized treatment of yaws, scabies food and drinks in West Africa, and in some parts of India (Gautam and Navneet, 2014). In the Malyagiri hills, a decoction made from 15 leaves each of *C. occidentalis* is used for bathing the new born to make the baby almost immune to skin diseases by the Tanla people in Dhenkanal district of Orissa (Singh*et al.,* 2016). The plant is also used to cure sore eyes, hematuria, rheumatism, typhoid, asthma and disorder of hemoglobin and is also reported to cure leprosy. An infusion of the bark is given for the treatment of diabetes (Verma *et al.,* 2010 and Chinnala *et al.,* 2017).

1.8.7 Pharmacological uses

Pharmacological studies displayed that *C. occidentalis* has an antioxidant, antibacterial, anti-inflammatory, analgesic, anti-pyretic, antimalarial, and hepatoprotective activities (Manikandaselvi *et al.,* 2016;

Yadav *et al.,* 2010 and Chukwujekwu *et al.,* 2007). It also exhibited significant antidiabetic activity in normal and alloxan induced diabetic rats (Singh *et al.*, 2016 and Kathirvel and Sujatha, 2012).

1.8.8 Toxicological studies

The toxicity of *C. occidentalis* has been documented in various animal experiments. The skeletal muscles, liver, kidney, and heart are all affected in large animals, rodents, and chickens. The brain's functions are frequently affected. At necropsy, skeletal muscle fiber necrosis and hepatic centrilobular necrosis are the most common gross lesions; renal tubular necrosis is less common. Biochemical abnormalities reflect muscle and liver cell necrosis. For mice and rats, the median lethal dose (LD_{50}) is 1 g/kg. The toxicity of *C. occidentalis* is mainly attributed to anthraquinones, their derivatives, and other alkaloids, although the exact poisons have yet to be discovered (Vashishtha *et al.,* 2009).

The toxicological effects of *C. occidentalis* on animals are ataxia, muscle weakness, stumbling, and body weight loss, eventually leading to death. Mechanism of *C. occidentalis* toxicity has been described as impairment of the mitochondrial function, including swelling, loss of mitochondrial matrix, myopathy, fragmented cristae and glycogen depletion. The stems and leaves of *Cassis occidentalis* cause considerable alterations in reproductive parameters, but they also cause abortive effects in females with doses of 0.25g and 0.5 g/kg/day, suggesting that Cassis stems and leaves are hazardous during pregnancy (Silva *et al.*, 2011).

Seeds of *C. occidentalis* were found to be toxic in pigs and rabbits. The histopathological examination of rabbits revealed that the heart and liver were the most affected organs with myocardial necrosis and centrolobular degeneration. The hepatic, skeletal muscles, and brain systems appear to be the most affected by *C. occidentalis* toxicity in

children. Poisoning from *C. occidentalis* pods causes fatal coma in the children of Western Uttar Pradesh. Cassia beans have a dose-dependent toxicity. *C. occidentalis* leaves may be slightly toxic as a concoction for liver ailments (Yadav *et al.*, 2010).

CHAPTER TWO

2. Materials and Methods

2.1 Plant material

Cassia occidentalis leaves were obtained from its natural habitat. They were collected in October, 2018 from Nyala City, Sudan. The plant material was taxonomically identified and authenticated by the botanists at Herbarium Unit, Department of Medicinal and Aromatic Plants, Traditional Medicine and Research Institute (MAPTMRI), National Center of Research (NCR), Khartoum, Sudan. A voucher (W-1995-63-MAPTMRI) of the plant specimen was prepared and also deposited in the herbarium. The leaves were cleaned and washed with distilled water and air dried at room temperature and then powdered using blender.

2.2 Plants extracts

Six hundred gram of the dried powder of *C. occidentalis* leaves was extracted using methanol98%. The methanolic extract was prepared using soxhlet apparatus. The extract was then distilled to dryness under a reduced pressure using Buchi rotary evaporator. The yield of the extract was measured and calculated (Harborne, 1984).

2.3 Phytochemical analysis

Methanolic extract of *C. occidentalis* leaves was subjected to preliminary phytochemical screening for the presence or absence of secondary metabolites. The screening include detection for the presence of alkaloids, flavonoids, tannins, saponins, sterols and triterpenes, cumarins and anthraquinones (Harborne, 1984). The analysis is based on the application of specific reagents to particular amounts of *C. occidentalis*

leaves methanolic extract and the identification of changes in solution colour.

2.3.1 Test for alkaloids

About 0.5g of *C. occidentalis* leaves methanolic extract filtrate was treated with Mayer's reagent (potassium mercuric iodide). Yellow deposit formation indicates the presence of alkaloids.

2.3.2 Test for flavonoids

Half gram of the extract was treated with few drops of lead acetate solution. Flavonoids are suggested by the formation of yellow precipitate colour.

2.3.3 Test of Tannins

Approximately 0.5g of methanolic extract was dissolved in distilled water, boiled for 5 minutes then 2 drops of $FeCl_3$ was added. Production of greenish precipitate indicate the presence of tannins.

2.3.4 Test for saponins

About 0.3g of the extract was shaken with 2 ml of water. Foam development which persists for 10 minutes indicates the presence of saponins.

2.3.5 Test of sterols and triterpenes

About 0.5 g of the extract was treated with chloroform and filtered. The filtrate was treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of blue pink to purple color was taken as an evidence of the presence of sterols (green to blue) and triterpenses (pink to purple) in the sample.

2.3.6 Test for cumarins

Approximately 0.2 g of the extract dissolved in 10 ml distilled water in test tube and a filter paper was attached to the test tube to be saturated with a vapor after spots of 0.5N-KOH were placed on a filter paper, then the filter paper was examined under UV light, the presence of coumrins was indicated when the spot adsorbed the UV light.

2.3.7 Test for anthraquinones

One gram of the powdered *C. occidentalis* leaves was boiled with 10 ml of 0.5 N-KOH containing 1ml of 3% hydrogen peroxide solution. The residue was extracted by shaking with 10ml of benzene. 5ml of the benzene solution was shaken with 3 ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of anthraquinones was proved when the alkaline layer was found to have assumed pink or red colour.

2.4 Antioxidant activity

The 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) radical scavenging assay was performed according to the method of Shimada *et al.* (1992) with some modification. In 96-wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37ºC. The concentration of DPPH was kept as 300 μM. The test samples were dissolved in DMSO (Dimethyl Sulphoxide), while DPPH was prepared in ethanol. The test samples were dissolved while DPPH was prepared in ethanol. After incubation, the decrease in absorbance was measured using multiplate reader spectrophotometer. The percentage of radical scavenging activity by samples was determined in comparison with a DMSO treated control group. Propyl gallate was used as a standard antioxidant agent. The analysis was run in triplicate**.**

2.5 Experimental animals

Adult Wistar albino rats (2 months' age) weighting between 82 – 123g were obtained from MAPTMRI. The rats were preserved in plastic cages in the laboratory animal house in MAPTMRI. They were fed with standard feed pellets and tap water *ad libitum*. The animals were acclimatized for one weeks before experimentation. The research was carried out in compliance with the Guiding for the Care and Use of Laboratory Animals, Sudan University of Science and Technology, College of Veterinary Medicine.

2.6 Experimental design

Seventy (70) healthy Wistar albino rats were divided into 7 groups of 10 rats each.

Group 1: Kept untreated as control for 8 days.

Group 2: Nephrotoxic control, rats injected with gentamicin at a dose of 80mg/kg intraperitoneally (IP) for induction of nephrotoxicity daily for 8 days.

Group 3: Standard drug, animals received gentamicin (80mg/kg) IP and standard silymarin at a dose of 100mg/kg orally, daily for 8 days.

Group 4: Low dose of the plant, rats injected with gentamicin at a dose of 80mg/kg IP with concurrent administration of *C. occidentalis* leaves methanolic extract at a dose of 200 mg/kg orally, daily for 8 days.

Group 5: High dose of the plant, rats injected with gentamicin at a dose of (80mg/kg) IP with simultaneous administration of *C. occidentalis* leaves methanolic extract at a dose of 400 mg/kg orally, daily for 8 days.

Group 6: Toxicity of low dose; rats given *C. occidentalis* leaves extract at a dose of 200 mg/kg orally, daily for 8 days.

Group 7: Toxicity of high dose, animals administered *C. occidentalis* leaves extract at a dose of 400 mg/kg orally, daily for 8 days.

2.7 Blood sample collection

Blood for serum extraction was collected in plain containers from the retro-orbital plexus under anaesthesia. Samples were collected at day 0 and day 8. They were kept in a refrigerator at -20°C until used for biochemical studies. Another blood samples were collected in EDTA tube for haematological studies.

2.7.1 Haematological methods.

Packed cell volume (PCV), haemoglobin concentration (Hb), red blood cells count (RBC), white blood cells count (WBC), Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were analyzed using automatic analyser (Humacount plus- Human GmbH Max- Planck-Ring21, D-65205 Wlesbaden, Germany).

2.7.2 Biochemical analysis:

Parameters including urea, creatinine, uric acid and total protein were measured by biochemical auto-analyzer (Mandary-autoanalyzer) using commercial kit.

2.8 Body weight of rats

Body weight of animal was recorded at day 0 and day 8 using standard scale.

2.9 Postmortem

At the end of the experiment, rats were sacrificed and the kidneys were examined.

2.10 Calculate relative kidney weight

The kidneys were isolated, weighed and relative weight of the kidneys were calculated from the ratio of organ weight to body weight (Piao *et al.*, 2013).

Relative kidney weight = (The organ wt. /Body wt.) × 100

2.11 Data Analysis

Statistical analysis was performed using software statistical package for science (SPSS) version 22. The data were analyzed using one-way analysis of variance (ANOVA) and compared by Post Hoc test (Duncan). The results obtained were considered significant at $p < 0.05$. The data were expressed as mean ±SEM (Mendenhall, 1971).

CHAPTER THREE

3. Results

3.1 Plant extraction

Cassia occidentalis leaves (600 g) was extracted with methanol (98%) using soxhelt apparatus. The yield of the extract is summarized in table 2.

3.2 Phytochemical analysis

Preliminary phytochemical screening of *C. occidentalis* leaves methanolic extract revealed the presence of tannins, saponins, coumarins, and alkaloid as active components and devoid of flavonoids and sterols. The phytochemical results of *C. occidentalis* methanolic extract are presented in table 3.

3.3 Antioxidant activity of methanolic extracts of *C. occidentalis* leaves.

C. occidentalis leaves metanolic extract exhibited moderate antioxidant activity (50 ± 0.04%) using DPPH radical scavenging assay. The propyl gallate used as a standard antioxidant agent showed high antioxidant activity (93 ± 0.01%). The results are shown in table 4.

3.4 Haematological parameters

There were no significant differences ($P > 0.05$) between group 1 (normal) and treated groups (group 2-7) on the haematological parameters during the period of experiment in day 0 and day 8. However, these values were fluctuated within the normal ranges in different groups. The results are presented in table 5.

3.5 Biochemical analysis

The levels of urea, creatinine, uric acid and total protein in control and treated groups were on the normal ranges in day 0. The levels of

creatinine, uric acid and total protein insignificantly change ($P > 0.05$) at day 8 in group 1 compared with group 3, 4, 5, 6 and 7.

However, the levels of urea were significantly increased ($P < 0.05$) in group 2 compared with group 1 and other treated groups. There were no significant differences ($P > 0.05$) in urea levels between group 1, 3, 4, 6 and7. Urea level in group 7 was significantly higher ($P < 0.05$) compared with group 1, but comparable with the levels of group 3, 4, 6 and 7as presented in table 6.

3.6 Body weight of rats

There were no significant differences ($P > 0.05$) in rats' body weights between group 1 and group 2, 3,4,5,6 and 7 during the period of experiment in day 0 and day 8. The results are presented in table 7.

3.7 Postmortem

No significant pathological changes were seen in experimental rats of group 1, 6 and 7. However, group 3, 4 and 5 showed enlarged kidneys while group 2 showed enlarged pales kidneys as presented in Fig. 2.

3.8 Relative kidney weight

The relative weight of kidneys was significantly increased in group 2 and in rats receiving the standard drug and plant extracts compared to control as shown in table 8. Rats that received low and high doses of the extract only showed insignificant change of kidney weights.

Table (2). The yield of *C. occidentalis* leaves methanolic extract.

Plant	Weight /g	Solvent	Yield (g)	Yield %
***C. occidentalis* leaves**	600 g	Methanol	19.24 g	3.21

Table (3). Phytochemical screening of methanolic extracts of *C. occidentalis* leaves.

Plant material	FL	TA	TR	SA	CO	AL	ST	AN
Leaves	-	+	-	+	+	+	-	-

Key words: FL= flavonoids, TA= tannins, TR= triterpenoids, SA= saponins, CO= coumarins, AL= alkaloid, ST= sterols, AN= anthraquinones. + found; - not found.

Table (4). Antioxidant activity of *C. occidentalis* leaves methanolic extract using DPPH radical scavenging assay

No.	Sample	%RSA ±SD(DPPH)
1	Leaves	50 ± 0.04
2	Propyl Gallate	0.01 ±93

Table (5). Haematological changes of rats administered methanolic extracts of *Cassia occidentalis* leaves, gentamicin and silymarin.

Groups	WBCs ($\times 10^3$/µl)		RBCs ($\times 10^6$ cells/µl)		Hb (g/dl)	
	Day 0	Day8	Day 0	Day8	Day 0	Day8
Group 1	6.60± 0.59	7.81± 1.25 [a]	7.22±0.39	7.24± 0.28 [a]	16.00± 0.55	15.36± 0.29 [a]
Group 2	7.11± 0.67	6.99± 1.18 [a]	6.81±0.14	6.63± 0.17 [a]	14.89± 0.55	14.66± 0.68 [a]
Group 3	7.13±0.78	7.02± 1.14 [a]	7.26 ±0.29	7.11± 0.38 [a]	15.92± 0.82	14.86± 0.49 [a]
Group 4	7.73±0.60	7.01± 1.53 [a]	6.79±0.53	7.27± 0.19 [a]	15.47± 0.65	15.63± 0.49 [a]
Group 5	7.73± 0.96	7.69± 1.58 [a]	7.52±0.24	7.16± 0.21 [a]	15.62± 0.40	15.46± 0.33 [a]
Group 6	8.13± 0.10	8.87± 1.22 [a]	7.46±0.18	7.31± 0.08 [a]	14.43± 0.22	15.00± 0.53 [a]
Group 7	7.73± 0.86	9.54± 1.97 [a]	7.31±0.12	6.63± 0.26 [a]	15.43± 0.76	14.62± 0.78 [a]

Groups	PCV%		MCV (fl)		MCH (pg)	
	Day 0	Day8	Day 0	Day8	Day 0	Day8
Group 1	39.10± 0.87	44.04± 1.52 [a]	56.10± 4.25	61.06± 1.13 [a]	23.19± 2.43	21.46± 1.05 [a]
Group 2	35.11± 1.23	39.01± 1.10 [a]	51.64± 1.30	58.99± 1.36 [a]	21.84± 0.72	22.09± 0.94 [a]
Group 3	38.56± 2.37	44.64± 2.02 [a]	52.60± 1.77	63.08± 1.66 [a]	22.32± 1.06	21.10± 1.32 [a]
Group 4	41.31± 1.44	44.90± 0.67 [a]	57.87± 3.82	61.02± 0.90 [a]	21.94± 1.45	21.21± 0.79 [a]
Group 5	40.94± 1.06	43.41± 1.33 [a]	54.64± 0.74	60.79± 0.96 [a]	20.88± 0.78	21.66± 0.69 [a]
Group 6	39.77± 1.32	45.08± 0.72 [a]	53.33± 0.73	61.80± 1.01 [a]	19.37± 0.65	20.48± 0.72 [a]
Group 7	39.96± 1.18	42.34± 0.64 [a]	54.67± 0.93	61.92± 0.90 [a]	21.00± 0.78	22.00± 0.80 [a]

Groups	MCHC (g/dl)		PLT ($\times 10^3$ /µl)	
	Day 0	Day8	Day 0	Day8
Group 1	41.02± 1.61	35.26± 1.66 [a]	540.00 ± 40.59	603.44± 32.18 [a]
Group 2	42.45± 1.13	37.60 ± 1.77 [a]	576.63± 31.89	632.57± 22.23 [a]
Group 3	42.16 ± 1.47	33.52 ± 1.84 [a]	569.40 ± 40.47	609.60 ± 18.49 [a]
Group 4	38.08± 1.35	34.82 ± 1.18 [a]	529.25 ± 57.62	561.00± 44.43 [a]
Group 5	38.23 ± 1.16	35.76± 0.96 [a]	536.89± 37.04	607.56± 39.97 [a]
Group 6	36.48 ± 1.53	33.28 ± 1.41 [a]	667.33 ± 43.78	706.17± 42.53 [a]
Group 7	38.46± 1.06	35.58± 1.05 [a]	641.56± 92.89	628.89± 65.11 [a]

Key: Not significant when compared with control P>0.05. Data are expressed as mean ±SEM (N =10).

Table (6). Biochemical changes of rats administered methanolic extracts of *C. occidentalis* leaves, gentamicin and silymarin.

	Urea (mg/dl)		Creatinine (mg/dl)	
Groups	**Day 0**	**Day8**	**Day 0**	**Day8**
Group 1	20.71± 7.75[a]	43.88 ± 1.88[c]	0.89± 0.08[a]	1.16 ± 0.11[a]
Group 2	28.29± 7.44[a]	110.86 ± 22.18[a]	1.07± 0.20[a]	1.66 ± 0.55[a]
Group 3	27.40± 9.21[a]	66.40 ± 6.50[bc]	1.07± 0.08[a]	1.06 ± 0.12[a]
Group 4	30.89± 8.27[a]	58.13 ± 4.89[bc]	0.90± 0.14[a]	1.04 ± 0.16[a]
Group 5	21.83± 8.50[a]	70.63 ± 8.24[b]	1.02± 0.16[a]	1.21 ± 0.09[a]
Group 6	14.67± 7.47[a]	51.50 ± 5.10[bc]	0.89± 0.08[a]	1.18 ± 0.06[a]
Group 7	16.00± 8.26[a]	55.67 ± 2.90[bc]	0.96± 0.05[a]	1.08 ± 0.06[a]

	Uric acid (mg/dl)		Total Protein (g/dI)	
Groups	**Day 0**	**Day8**	**Day 0**	**Day8**
Group 1	4.42± 0.48 [a]	3.54 ± 0..37 [a]	7.24 ± 0.43 [a]	6.48 ±0.50 [a]
Group 2	4.51± 0.38 [a]	2.87 ± 0.40 [a]	6.94 ± 0.73 [a]	6.76 ± 0.52 [a]
Group 3	6.38 ± 1.08 [a]	3.70 ± 0.72 [a]	6.38 ± 0.38 [a]	6.30 ± 0.24 [a]
Group 4	6.07± 1.04 [a]	3.26 ± 0.49 [a]	6.16 ± 0.36 [a]	6.10 ± 0.31 [a]
Group 5	5.88± 1.48 [a]	3.16 ± 0.74 [a]	5.87 ± 0.23 [a]	5.56 ± 0.21 [a]
Group 6	3.20 ± 1.19 [a]	4.22 ± 0.88 [a]	6.98± 0.63 [a]	6.13± 0.24 [a]
Group 7	2.65 ± 0.86[b]	4.14 ± 0.47 [a]	6.80 ± 0.17 [a]	6.66 ± 0.80 [a]

Key: Values are expressed as mean ± SEM, means within the same column with different superscripts are significantly different at $P< 05$ (N =10).

Table (7). Change of body weight in rats administered methanolic extracts of *C. occidentalis* leaves, gentamicin and silymarin.

Groups	Body weight (Mean ± SE) Days	
	Day 0	**Day8**
Group 1	110.89 ± 5.14	112.89±5.37 [a]
Group 2	119.13 ±12.87	107.57 ± 6.0 [a]
Group 3	112.17 ±8.92	113.33 ± 8.69 [a]
Group 4	114.22 ± 5.98	112.89 ± 5.52 [a]
Group 5	114.56 ± 6.311	113.22 ± 5.76 [a]
Group 6	121.67 ± 8.81	119.50 ± 9.22 [a]
Group 7	110.56 ± 6.03	109.56 ± 4.58 [a]

Key: Not significant when compared with control P>0.05. Data are expressed as mean ±SEM (N =10).

Table (8). Relative kidney weights after administration of methanolic extracts of *C. occidentalis* leaves, gentamicin and silymarin.

Groups	Relative kidney weight (Mean ± SE)	
	Right (g)	**Left (g)**
Group 1	$0.43 \pm 0.01^{b\,c}$	0.46 ± 0.02^{b}
Group 2	0.54 ± 0.01^{a}	0.53 ± 0.01^{a}
Group 3	0.53 ± 0.03^{a}	0.54 ± 0.03^{a}
Group 4	0.56 ± 0.01^{a}	0.56 ± 0.01^{a}
Group 5	$0.51 \pm 0.03^{a\,b}$	0.54 ± 0.01^{a}
Group 6	0.40 ± 0.05^{c}	0.45 ± 0.03^{b}
Group 7	$0.44 \pm .02^{c}$	$0.44 \pm .02^{b}$

Key: Values are expressed as mean ± SEM, means within the same column with different superscripts are significantly different at $P< 05$ (N =10).

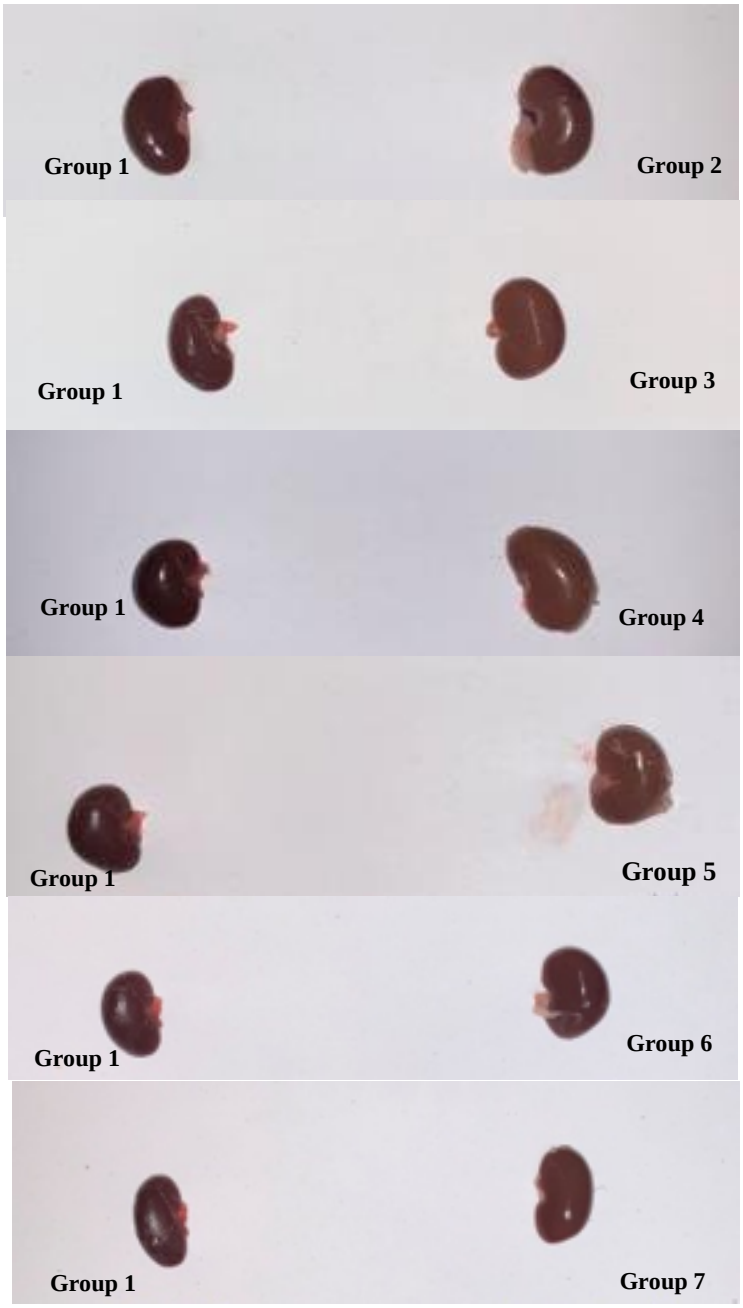

Fig.2. Pathological changes of kidneys in rats administered methanolic extract of *C. occidentalis* leaves, gentamicin and silymarin.

CHAPTER FOUR

4. Discussion

The plant *C. occidentalis* has been widely used in traditional medicine in the treatment of liver and urinary tract ailments (Manikandaselvi *et al.,* 2016 and Silva *et al.,* 2011). In the present study, the methanolic extract of *Cassia occidentalis* (sorib) leaves was evaluated for its antioxidant and nephroprotective activities using DPPH radical scavenging assay and gentamicin induced nephrotoxicity in albino rats respectively.

Nephrotoxicity caused by drugs recognized as acute kidney damage and chronic kidney disease (Aiswarya, *et al.,* 2018). Gentamicin is an aminoglycosides antibiotic. It has been reported to produce nephrotoxicity even at normal therapeutic dose level (Srivastava *et al.,* 2018). The nephrotoxicityof gentamicin characterized by elevation of urea, creatinine, and uric acid levels with marked decreases in glomerular filtration rate, tubular necrosis, dilatation and degeneration of tubules (Aiswarya, *et al.,* 2018; Hussain *et al.,* 2012 and Lakshmi *et al.,* 2009).

The mechanisms of gentamicin renal toxicity are not fully known. Several studies suggest that gentamicin overdose stimulates the formation of reactive oxygen species in renal tissues, by decreasing renal glomerular filtration as well as antioxidant enzymes, and increases plasma creatinine, urea, lipid peroxide formation, infiltration of inflammatory cells, and sub sequent release of pro-inflammatory cytokines (Heidarian *et al.,* 2017).

In this study, the administration of gentamicin at a dose of 80 mg/kg to nephrotoxic group rats produced a deterioration in renal function, as shown by an increase in blood urea and pathological changes of kidneys in gentamicin group, which is consistent with previous results of Aiswarya *et al.* (2018). Gentamicin promotes generations of hydrogen peroxide and free

oxygen radicals. Reactive oxygen species (ROS) can cause cell damage and necrosis through several mechanisms including, peroxidation of membrane lipids, denaturation of proteins and DNA damage (Suji Arivazhagan, and Vimalastalin, 2014). It has also been reported that gentamicin alters the basolateral membrane and mitochondria enhancing production of free radicals and lipid peroxidation of renal cortex (Aiswarya *et al.,* 2018).

Creatinine and uric acid levels were not significantly affected in the present study. Moreover, gentamicin is known to produce reactive oxygen species in the kidney, which is associated to an increase in lipid peroxidation and a reduction in antioxidant enzyme activity (Alarifi *et al.,* 2012). Our study proved this report.

Simultaneous treatment of methanolic extract of *C. occidentalis* leaves and gentamicin provided marked nephroprotection against gentamicin induced renal damage in rats as evidenced by significant reduction in biochemical markers, particularly urea levels. The rate of urea generation exceeds the rate of clearance in renal diseases, causing an increase in serum urea. Creatinine is derived from endogenous sources on a regular basis through tissue creatinine breakdown. The rise in blood creatinine was associated to structural damage in the kidneys (Alarifi *et al.,* 2012). However, the level of creatinine in this study was increased but it was not significant.

The study also displayed that C. *occidentalis* leaves may have antioxidant activity. Several studies have shown antioxidant potential of *C. occidentalis* leaves (Ntchapda *et al.,* 2015 and Arya*et al.,* 2011).

When comparing the low dose of sorib (200 mg/kg) to the large dose (400 mg/kg), the low dose appears to provide superior nephron-protection effect. However, haematological parameters were not affected in all treated group.

In this study, tannins, saponins, coumarins, and alkaloid were identified as active components in the methanolic extract of *C. occidentalis* leaves. However, flavonoids, sterols and anthraquinones were not found. Nuhu and Aliyu, (2008) stated that the leaves of *C. occidentalis* contain tannins, saponins, and flavonoids and devoid of alkaloids. Phytochemicals such as, alkaloids have been shown to reduce lipid peroxidation in isolated tissues by acting as antioxidants (Palani *et al.,* 2009).

Some nephroprotective plants have been found to reduce the harmful effects of nephrotoxic drugs in experimental animal models due to their powerful anti-oxidant or free radical scavenging activities such as *Pimpinella anisum* (Aiswarya, *et al.,* 2018), *Rosa damascena* flowers, *Cichorium intybus* roots (Khaliq *et al.,* 2015) and *Daucus carota* (Sodimbaku *et al.,* 2016).

CONCLUSION AND RECOMMENDATIONS

Conclusion

To conclude, this study displays that an orally taken methanolic extract of *C. occidentalis* leaves can protect kidney against gentamicin-induced nephrotoxicity in rats. It also indicates that the plant's phytoconstituents or antioxidant activity may responsible for the observed protective effects. This supported the folklore use of the plant in renal disorders.

Recommendations

- More studies are required to confirm the nephroprotective activity of this extract.
- Further investigations should be performed to determine the exact phytoconstituent(s) responsible for the nephroprotective effect of *C. occidentalis* leaves methanolic extract.
- Further investigations should be done to assess the toxicological effects and other biological activities of *C. occidentalis* leaves methanolic extract.

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Appendixes

Appendix 1

Identification of *C. occidentalis* leaves certificate .