Effect of *Moringa oleifera* on Kidney Disease: A Review

By:

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of B.Sc. in Pharmacy.

School of Pharmacy
Brac University
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Declaration

I hereby declare that

1. The thesis presented is my original work completed while pursuing the Bachelor of pharmacy at Brac University.
2. The thesis does not incorporate anything previously published or generated via any third party, unless it is correctly cited through extensive & exact citing.
3. The paper does not include any content which has been authorized or presented for a degree or certificate from another institution or university.
4. All significant sources of assistance have been recognized.

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Approval

The thesis titled "**Effect of *Moringa oleifera* on Kidney Disease: A Review**" is submitted by: Muntasir Al Muhaimeen (18146060) has been approved as adequate for partial completion of the requirements for the Bachelor of Pharmacy degree.

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Ethics Statement

This paper does not include any animal or human experiments.

Abstract

The prevalence of kidney disease has been identified as a major public health issues on a global scale. The number of people with end-stage kidney disease (ESKD) who require renal replacement therapy is expected to be between 4.902 and 7.083 million worldwide per year. Recent studies have shown that *Moringa oleifera* has a number of positive effects on kidney functions including reducing serum creatinine level, blood urea nitrogen, kidney weight etc. In this review the goal was to find the factors that lead to kidney disease. And how *Moringa oleifera* can play a role in preventing kidney disease by eradicating or neutralizing these factors. From the review articles it has been evident that the antioxidant, anti-inflammatory and anti-fibrotic properties of *Moringa oleifera* helps as a remedy for kidney disease. However, all these animal studies highlight the necessity of conducting clinical trials of *Moringa oleifera* on kidney disease.

Keywords: *M.oleifera*, kidney disease, creatinine, clinical uses, diabetes, high blood pressure, inflammation, oxidative stress, fibrosis, bioactive compounds.

Dedication

I want to dedicate this project to my respectable supervisor Dr. Sharmind Neelotpol, Associate Professor, School of Pharmacy, Brac University for her continuous and relentless guidance throughout my project. I want to dedicate this project to my family as well for their unwavering support and motivation to fulfill my desired goal.

Acknowledgement

I would like to proceed by thanking the Almighty Allah who is the source of our strength and knowledge which have enabled me to complete this project with full diligence.

I would like to showcase my deepest and most sincere gratitude to my project supervisor, Dr. Sharmind Neelotpol, Associate Professor, School of Pharmacy, Brac University, whose expertise, constant guidance, sincere monitoring, and motivational approaches in every sphere have inspired me to conduct this project efficiently. I sincerely express my deepest appreciation and admiration for her patience and she provided a sense of direction whenever I encountered complications throughout this phase. I also wanted to acknowledge the support of all my faculty members who helped me in need.

Lastly, I would like to thank my parents for their constant support and encouragement that further pushed me to work harder and encouraged me to face the difficulties during this time frame.

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List of acronyms:

- MO: *Moringa oleifera*
- NAFLD: Non-alcoholic fatty liver disease
- AKI: Acute kidney damage
- CKD: Chronic kidney damage
- A549: Adenocarcinomic human alveolar basal epithelial cells
- MCF-7: Michigan Cancer Foundation-7(cancer cell)
- BUN: Blood urea nitrogen
- MDA: Muscular Dystrophy Association

Chapter 1

1.1 Introduction

One of the world's most important health issues is kidney disease which means that the kidneys are unable to filter blood the way they should. There are two basic categories of kidney disease: acute kidney disease and chronic kidney disease. Short-term kidney disease usually leads to full recovery, although it can raise a chance of developing a later-life chronic kidney disease (Bindroo et al., 2022).

The primary causes responsible for kidney diseases are oxidative stress, apoptosis, fibrosis, & inflammation. Unfortunately, there are currently no individual drugs that could be used to treat renal problems. As a result, it is critical to find a medicine that could treat this condition and has fewer adverse effects. In the search for medicine, *Moringa oleifera* (*M.oleifera)* has shown positive results in multiple tests such as: reducing serum creatinine level, reducing blood urea nitrogen, antioxidant and anti-inflammatory properties to eradicate the ongoing kidney disease throughout the world (Vergara-Jimenez et al., 2017).

Moringa leaves have anti-inflammatory properties because of the presence of phenolic glycosides, which helps diabetes patients in avoiding circulatory problems (Shourie, 2016).

Moringa includes a number of bioactive phytochemicals that has been documented in its seeds, roots, flowers, & fruits. These substances include vanillin, flavonoids, omega fatty acids, saponin, ascorbates, carotenoids, kaempferol, tocopherols & quercetin. In addition, bioactive components available in *M.oleifera* protect against kidney disease (Vergara-Jimenez et al., 2017).

Furthermore, *M.oleifera* is thought to be a promising medicinal plant for the treatment of neurodegenerative conditions. Different research studies found the neuroprotective effects of *M.oleifera* on Parkinson's disease, Dementia and Alzheimer's disease (Ghimire et al., 2021).

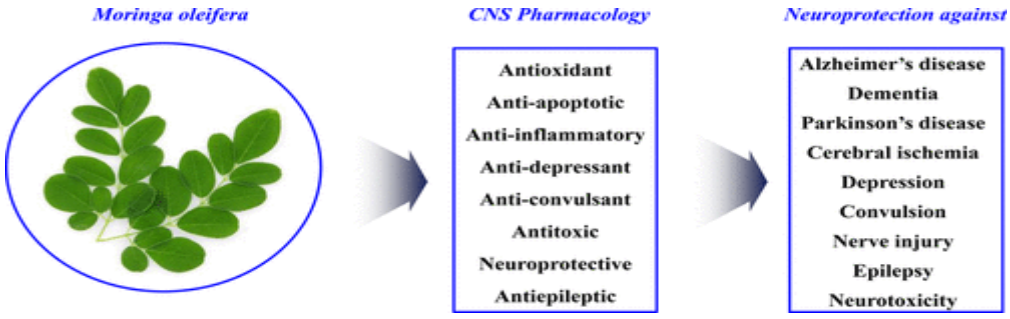

Figure 1: *Pharmacological properties of M.oleifera (Source:Ghimire et al., 2021).*

Figure 1, manifests that *M,oleifera* has the potential to act as an antioxidant, anticancer, anti-inflammatory, anti-diabetic, & antibacterial agent (Ghimire et al., 2021).

Moringa oleifera, also known as the Shojne, is a native of the Himalayan foothills in Bangladesh and India. The Moringa is a plant that is grown in Florida, Hawaii, India, Africa, Pakistan, the West Indies, the Philippines, Jamaica, and Cuba. This tree will flourish everywhere that has tropical or subtropical climates. It has over 13 different species, and all of its parts are consumed or utilized as a medicine in different regions of the world. Some regions eat seeds the same way they eat peanuts. Salads frequently contain leaves, which are loaded with vitamins and antioxidants and have a very high nutritional value (Patterson, 2021).

1.2 Aim of the study

The overarching aim of this study was to evaluate the effect of *Moringa oleifera* on kidney disease.

1.3 Objectives of this study

The objectives of this study were to:

- evaluate the mechanism of action of *M. oleifera* on kidney disease;
- find out the anti-inflammatory and antioxidant activities of *M. oleifera* on kidney disease;
- explore the evidence of the role of *M. oleifera* on the reduction in creatinine level.

Chapter 2

Methodology

This study was brought up to date by searching for the key terms "Factors causing kidney disease" & "*M.oleifera* on kidney disease & creatinine level" in internet research databases such as Google Scholar & PubMed. The following sets of validation checks & scrutiny analyses of recently published literature were utilized in the investigation of this study.

We searched for material utilizing the keywords on topics such as 'kidney disorders,' 'oxidative stress,' & 'inflammation,' as well as 'fibrosis,' & '*Moringa oleifera*' in various online databases. Google Scholar and PubMed were some of the databases that we used. Some of the items were eliminated from consideration through the use of automated search engines, while others were examined manually. We did not include any articles that were first published in a language other than English. The following types of writing were not considered for inclusion in this review: expert opinions, and letters to the editor.

Chapter 3

Discussion

3.1 Factors that lead to kidney disease

Kidneys excrete wastes and extra fluid from our blood. Kidney diseases are of two types - acute kidney disease which is short term and chronic kidney disease which can be lifelong. Factors that lead to kidney disease are mostly diabetes and high blood pressure. There are more factors that also lead to kidney disease. Such as: inflammation, oxidative stress and fibrosis (Luyckx et al., 2017).

These factors are described below with an explanation of how they affect the kidney.

3.1.1 Diabetes

The glomerulus in our kidneys are damaged by elevated blood glucose. High blood glucose damages the kidneys over time, making it difficult for it to eliminate the waste products and additional fluid from the blood. First sign of renal impairment is the presence of protein in the urine brought on by diabetes. When filters are compromised, albumin, leaks out from the blood into the urine. In a healthy kidney this event does not take place (Luyckx et al., 2017).

About 40% of people suffering from diabetes develop diabetic kidney disease, which is the primary cause of CKD worldwide. Though end-stage renal disease (ESRD) is also well-known for complication of diabetes. Diabetes induces hemodynamic, metabolic, inflammatory and fibrotic pathophysiological factors which lead to kidney failure. The majority of people pass away from infections and cardiovascular disorders before requiring kidney replacement therapy. Glomerular hyperfiltration, increasing albuminuria, falling GFR, and finally end-stage renal disease (ESRD)

are all features of natural course of diabetic kidney disease. Glomerular hypertrophy, tubulointerstitial inflammation, glomerulosclerosis and fibrosis are all results of the metabolic alterations brought on by diabetes (Alicic et al., 2017).

Figure 2 below showed a graphical representation of how diabetes affects the kidney.

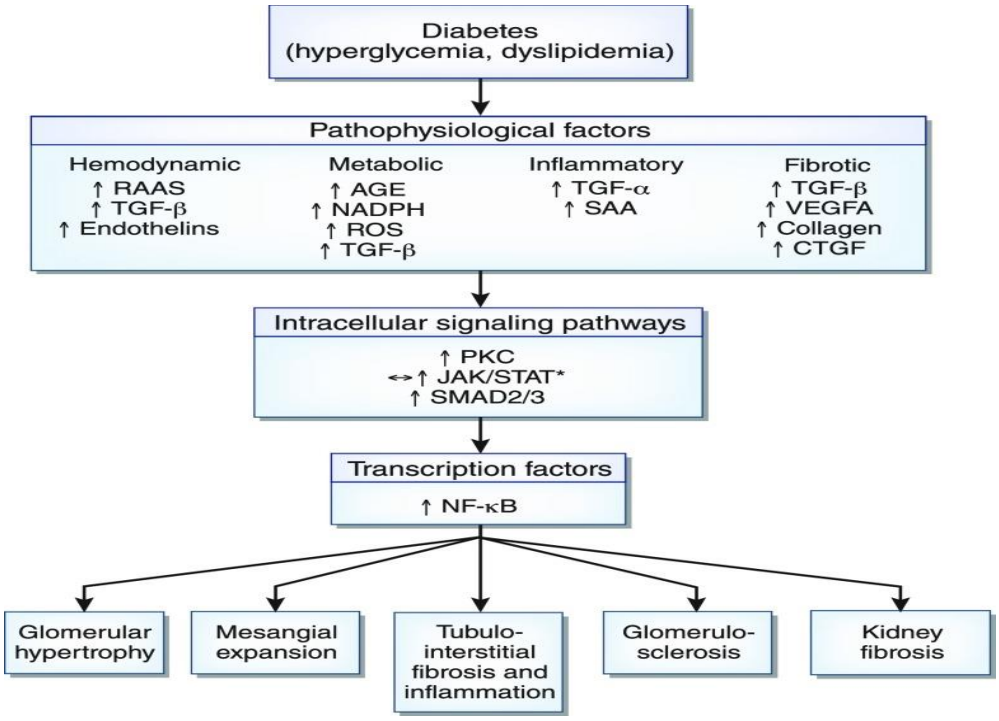

Figure 2: *Different networks and pathways involved in the instigation and development of diabetic kidney disease (DKD) (Source:Alicic et al., 2017).*

In figure 2, we can see that diabetes induces hemodynamic, metabolic, inflammatory and fibrotic pathophysiological factors. These factors in return activate intracellular signaling pathways. As a result of these events, nuclear factor κB an essential transcription factor is increased which eventually causes renal inflammation which leads to kidney fibrosis.

3.1.2 High blood pressure

The blood vessels become narrower due to high blood pressure, which affects the kidneys. Blood arteries in the kidneys may get damaged and stop functioning normally. When this happens, the kidneys lose their ability to cleanse wastes and extra fluid from the body. With too much fluid in the kidneys a deadly cycle can be created, which increases the blood pressure even higher and further damage the kidneys (Luyckx et al., 2017).

To regulate blood pressure in the body there is a system called RAAS (renin-angiotensin-aldosterone system). During high blood pressure in the body, the RAAS system starts to dysfunction by promoting oxidative stress in the brain which causes kidney disease in the long run (Takahashi et al., 2011).

Figure 3 illustrates how high blood pressure causes kidney disease.

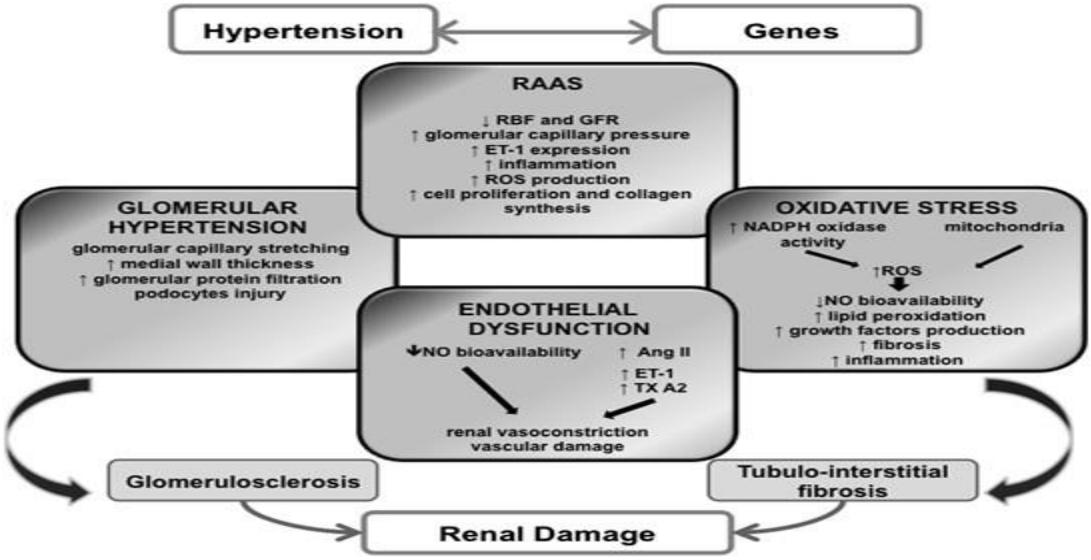

Figure 3: *Events causing hypertensive renal damage (Source:Mennuni et al., 2013).*

Figure 3 shows how hypertension causes kidney disease. As hypertension deactivate the RAAS system in the kidney, it leads to glomerular hypertension and increases oxidative stress. The after events of these are glomerulosclerosis and tubulointerstitial fibrosis which causes kidney damage.

3.1.3 Inflammation

The vasculature or tissues may become inflamed in response to numerous stimuli. Patients with kidney disease have chronic and acute pro-inflammatory states. In individuals with ESRD and CKD, there are numerous mediators of inflammation, such as atherosclerosis, malnutrition, etc. By releasing cytokines and increasing the activity and production of adhesion molecules, it promotes T cell adherence and migration to the interstitium and subsequently drawing the pro-fibrotic substances. Thus, inflammation aids in the progression of CKD (Silverstein, 2009).

Elevated production of oxidative stress, proinflammatory cytokines, acidosis, recurring and persistent infections and altered adipose tissue metabolism, intestinal dysbiosis are only a few of the variables that contribute to chronic inflammation in kidney disease. Systemic or intrarenal inflammation fuels the creation of a variety of tubular toxins, including reactive oxygen species (ROS), which causes tubular damage, nephron dropout, and the start of chronic kidney disease (CKD).

Circulating proinflammatory cytokines stimulate intrarenal micro vessels, especially leukocytes and endothelial cells, which causes a localized increase in proinflammatory substances and ROS. The glycocalyx layer is disturbed and the cell-surface adhesion molecules are impacted by these processes. Additionally, the coagulation system's activation, receptor-mediated vasoreactivity, and endothelial barrier function are impaired. These changes generated by inflammation can result in nephron failure and irreversible tubular damage (Mihai et al., 2018).

Although there have been recent improvements in the treatment of end-stage renal disease (ESRD) and chronic kidney disease (CKD), morbidity and death remain notably high in these individuals. Persistent, low-grade inflammation has been identified as a significant contributor to the CKD

scenario, causing fibrosis and the loss of renal function, and is a critical factor in the etiology, progression, and consequences of the illness (Mihai et al., 2018).

3.1.4 Oxidative stress

Oxidative stress results from a contrast between the formation of free radicals, which is frequently enhanced by type 2 diabetes mellitus, malfunctioning mitochondria that are brought on by aging, inflammation, and weakened antioxidant defenses. Changes in how cells handle oxidants have an impact on cellular signaling downstream and in the kidney, encouraging renal apoptosis, senescence, diminished cell regeneration capacity, and fibrosis (Small et al., 2012).

Oxidative stress at the renal level can result in renal ischemia, cell death, lesions to glomeruli and provoking the existing severe inflammatory processes. High levels of oxidative stress in the early stages of CKD have already been identified. These levels rise in tandem with the development of ESRD and are further aggravated in HD patients. When compared to uremic patients who are not on peritoneal dialysis (PD), ESRD patients exhibit more oxidative stress (but lower, when compared to HD patients). Moreover, it has been demonstrated that both HD and PD increase oxidative processes, which elevates the level of oxidative stress. Furthermore, oxidative stress could continue even after a kidney transplant (Rapa et al., 2019).

Reactive oxygen species (ROS) are further able to exacerbate the inflammatory response by inducing pro-inflammatory mediators such as NF-κB (nuclear factor kappa), whereas oxidative stress has also been connected to the generation of highly reactive intermediates during inflammation. The natural production of pro-oxidative agents by cells, which play vital defensive roles, is inhibited by enzyme systems such as glutathione and other antioxidants (also known as scavengers) because of their capacity to scavenge free radicals. ROS are primarily created in the kidneys by the mitochondrial respiratory chain and enzymes like NADPH oxidase (NOX).

Oxidative stress, which worsens vascular function and encourages fibrosis, is mostly caused by the many NOX isoforms, including NOX1, NOX2, and NOX4 (Rapa et al., 2019).

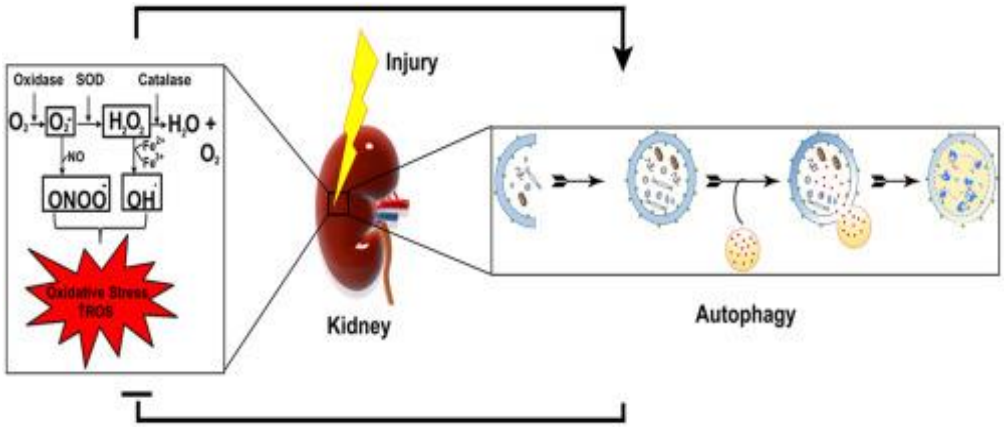

Figure 4: *Oxidative stress in Kidney (Source:Sureshbabu et al., 2015).*

In figure 4, it is illustrated how ROS causes oxidative stress in the kidneys that leads to autophagy and eventually cell death.

3.1.5 Fibrosis

The phenomena kidney fibrosis means excessive deposition and production of extracellular matrix (ECM) proteins primarily in kidney interstitium. Thus, resulting in impairment of renal function, structural damage, and eventually end-stage renal disease (ESRD) (Lee et al., 2018).

The innate and adaptive immune systems are involved in complicated inflammatory processes that characterize the early phases of the fibrotic process. Both M1 and M2 reparative macrophages are necessary for the initial inflammatory stage of the wound healing response. In distinction to these acute inflammatory reactions, fibrosis also occurs from chronic inflammation as a result of an immune response that lasts for many months, in which tissue remodeling, inflammation, healing processes take place simultaneously. Therefore, the fibrosis process is frequently started by persistent inflammation, a CKD feature (Panizo et al., 2021).

It is common knowledge that inflammation in the kidneys serves as the ignition for the start of renal fibrosis. The release of infiltration of inflammatory cells, cytokines and afterwards epithelial to mesenchymal transition (EMT) cause renal fibrosis and failure in both acute and chronic kidney damage. The epithelial growth factor receptor (EGFR) is activated during tubular healing processes. Even while its acute activation is advantageous in the initial phases of kidney damage, its persistent activation causes renal fibrosis. As a result of this activation, TGF-ß1 expression is increased, which drives the proliferation of interstitial myofibroblasts and causes the release of collagen and other ECM proteins, resulting in interstitial fibrosis and functional failure of nephrons (Panizo et al., 2021).

In figure 5, the difference between a normal kidney and a fibrotic kidney is shown.

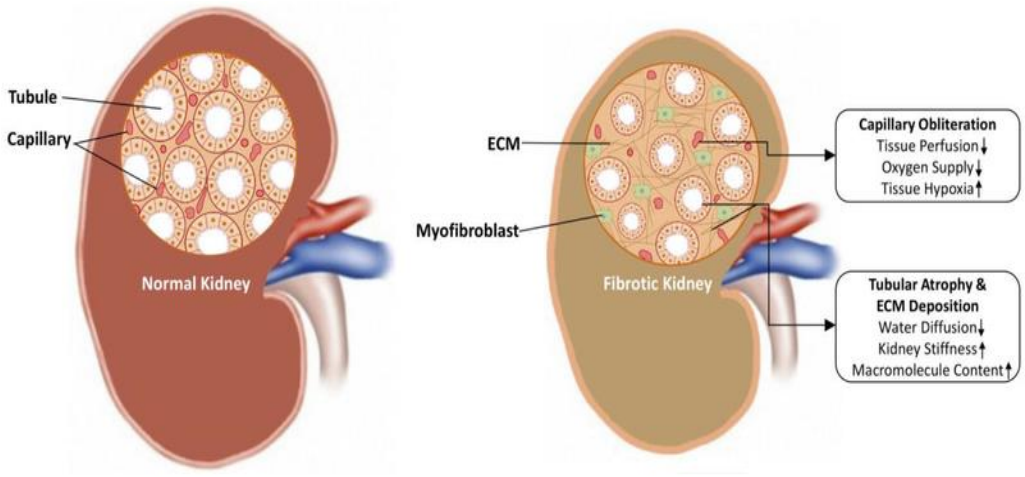

Figure 5: *Normal kidney vs fibrotic kidney (Source:Jiang et al., 2019).*

In figure 5, the difference between normal and fibrotic kidney is shown. In a fibrotic kidney the presence of myofibroblast produces a significant amount of ECM substances, such as type I/III collagens and fibronectin, which may later develop into fibrosis. Several alterations in the functional, molecular, & mechanical characteristics of the kidney are brought on by renal fibrosis, which frequently coexists with, kidney shrinkage, tubular atrophy, & vascular obliteration.

Vascular rarefaction reduces oxygen delivery and renal perfusion, resulting in tissue hypoxia. Water molecule movement is restricted by ECM deposition and accumulation, tubular atrophy, and a rise in kidney stiffness and macromolecule content (Jiang et al., 2019).

3.1.6 Other pathologies that are linked to kidney disease

Because autophagy plays such a crucial part in kidney physiology as well as homeostasis, the way in which it is regulated is one significant factor in determining renal disorders. Mitochondrial damage can be caused by AKI or CKD, but it can also be caused by certain stressors, which can lead to an accumulation of damaged mitochondria. The elimination of ROS-producing mitochondria by autophagy provides a defense mechanism to the kidney against damage. Apoptosis or programmed cell death is a phenomenon in which cells are eliminated by a mechanism that is under strict administrative control. This is a complex procedure that is energy-reliant. It causes the development of AKI & even organ failure. Ischemia & reperfusion, abbreviated as I/R, is a process that causes the kidney to undergo apoptosis or necrosis & results in the loss of tubular cells. Apoptosis is caused by tumor necrosis factor-alpha (TNF-a), which is expressed on the cell surfaces of renal tubular cells & is known as a "death receptor." Apoptosis is also caused by the formation of reactive oxygen species that occurs when renal disease is present (Akter et al., 2021).

In rats that had been treated with $CoCl_2$ (Phosgene: A toxic chemical compound), apoptosis was induced by TNF-, which also enhanced the development of apoptosis-related molecules, which were then down-regulated by an extract of *M.oleifera* made from ethanol. The development of caspase-9, the parent of caspase-3, was inhibited by leaf extract when it was given at a concentration of 300 mg/kg of body weight. This resulted in apoptosis. In ML-induced rats, the

ethanol extract of *M.oleifera* caused Bcl-2(B-cell leukemia/lymphoma 2 protein) to be up-regulated, which had the effect of inhibiting apoptosis. This was accomplished by stopping the release of cytochrome c & preventing caspase activation. Additionally, the synthesis of TIMP-1, a protein that plays a role in renal fibrosis & death, was inhibited by *M.oleifera* (Akter et al., 2021).

3.2 Evidence of Using *M.oleifera* for the Treatment of Kidney Diseases:

Kidney disease is most frequently brought on by both types of diabetes, type 1 and 2. Diabetes mellitus patients gradually experience circulation issues that result in multiple organ ailments. A major reason for this is chronic inflammation and the production of cytokines (proteins that regulate inflammation) found in diabetes. Moringa leaves have anti-inflammatory qualities because of the phenolic glycosides they contain, which helps diabetes patients avoid circulatory problems. Vascular damage brought on by elevated oxidative stress is a common complication of diabetes mellitus. The vulnerability of organs like the kidney to injury rises as the number of free radicals increases. Bioactive compounds found in moringa leaves have antioxidant qualities and work against free radicals to stop oxidative damage. This plays significant role in managing diabetes and its consequences (Shourie, 2016).

M.oleifera includes a variety of bioactive phytochemicals, including isothiocyanates; carotenoids, flavonoids, polyphenols, alkaloids, & triterpenoids, campesterol, moringyne, terpenoids, monopalmitin, & di-oleic triglyceride, stigmasterol, - vitamin A, sitosterol & avenasterol. Roots, and fruits of the *M.oleifera* plant all contain trace amounts of these beneficial compounds. These phytochemicals contain therapeutic qualities that have been found to be beneficial as anti-inflammatory agents, anti-carcinogenic agents, & antioxidants. They also have antibacterial effects. Additional research is necessary to investigate the role of bioactive phytochemicals,

particularly in the context of kidney disorders. The figure below shows the Protective mechanisms of *M.oleifera* against kidney injury. (Vergara-Jimenez et al., 2017)

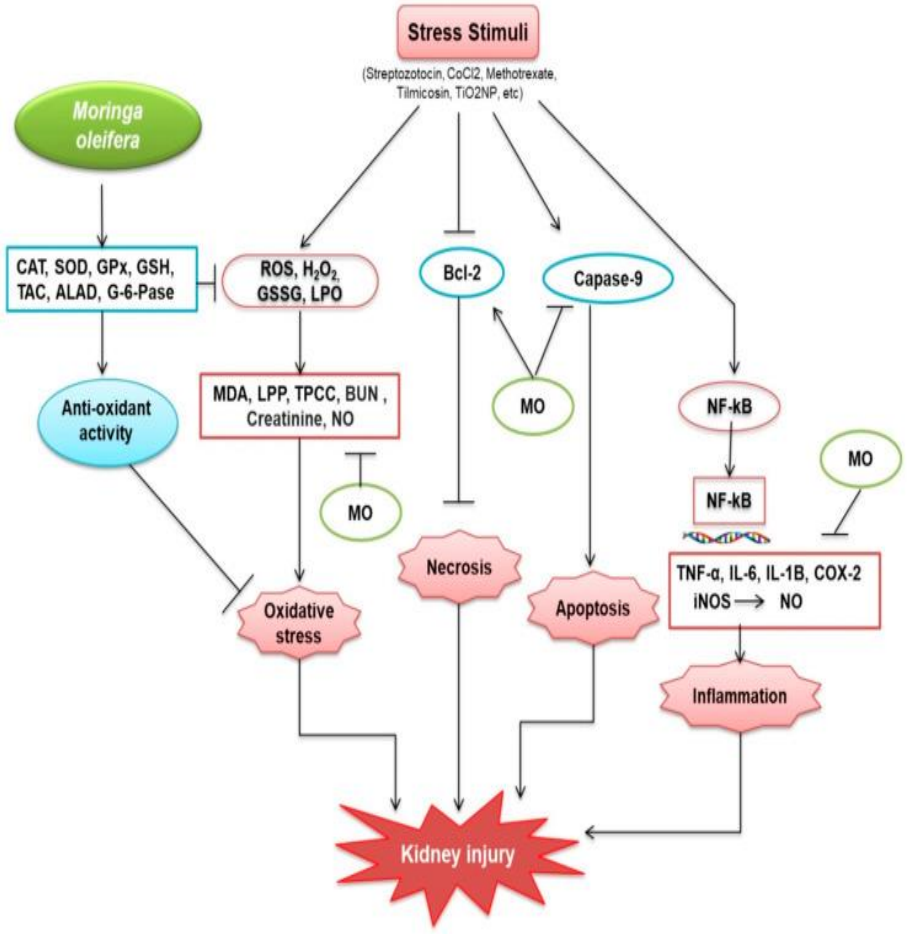

Figure 6: *Defense mechanisms of M.oleifera in Kidney injury (Source:Akter et al., 2021).*

In figure 6, it is shown that, ALAD (delta-amino levulinic acid dehydratase), G-6-Pase, SOD (superoxide dismutase), TAC (total antioxidant capacity), GPx (glutathione peroxidase), CAT (catalase), GSH (glutathione), & total antioxidant capacity (TAC) were all upregulated by *M.oleifera*. GSH, which is a non-protein thiol that inhibits free radicals. The oxidative stress state is suppressed by GSH. *M.oleifera* also inhibited MDA (Malondialdehyde), LPP (Lipoma-preferred

partner), BUN (blood urea nitrogen), Creatinine, and NO (nitric oxide) to regulate oxidative stresses brought on by ROS, H_2O_2. Bcl-2 which is also known as lymphoma 2 protein was linked to the inhibition of necrosis induced by *M.oleifera* and was similarly produced by stress stimuli. Caspase-9, which is a protein implicated in the production of caspases, was not expressed when *M.oleifera* was present. Stress-related stimuli also led to an increase in CRP expression after NF-kB inflammation. (Akter et al., 2021).

M.oleifera also exhibits a wide range of pharmacological attributes, all of those can be traced back to the existence of its bioactive components in the plant. *M.oleifera* shown pharmacological promise against a number of hypothesized contributors to kidney disease, including inflammation, oxidative stress, & other disorders (Akter et al., 2021). The possible protective benefits of *M.oleifera* towards renal disease risk factors are discussed below, as shown in Figures 7 & 8.

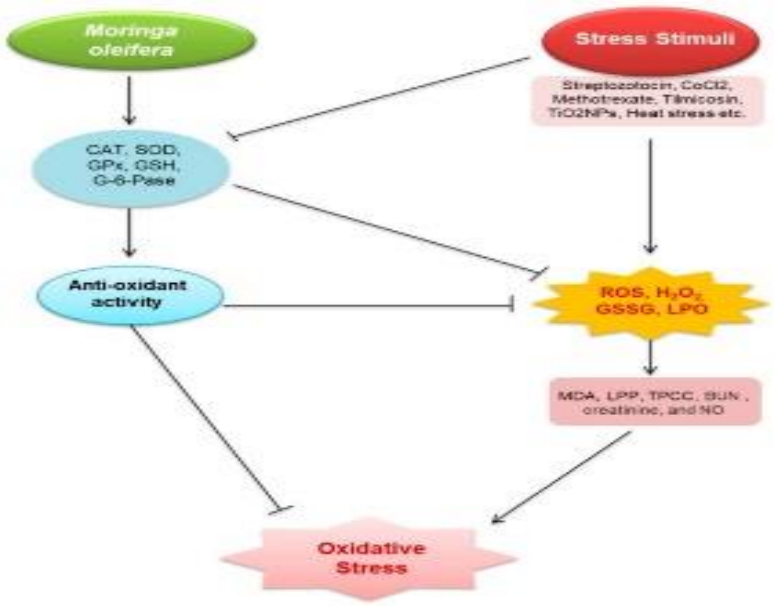

Figure 7: *Effects of M.oleifera on the protection of the kidneys against oxidative stress (Source:Akter et al., 2021).*

Figure 7 describes how *M.oleifera* protects the kidney against oxidative stress. Stress stimuli (methotrexate, streptozotocin, tilmicosin, NPs, $CoCl_2$.TiO_2, acetaminophen (APAP), *Salmonella*,

& glycerol) elevated MDA (malondialdehyde), TPCC (total protein carbonyl content), lipid peroxidation products (LPP), NO, creatinine, & BUN production by triggering reactive oxygen species (ROS), H_2O_2, glutathione disulfide (GSSG), & lactoperoxidase (LPO). Oxidative stress emerged as a result of these events. *M.oleifera* induced models, on the other hand, increased the expression of catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione (GSH), delta-amino levulinic acid dehydratase (ALAD), total antioxidant capacity (TAC), & G-6-Pase, which then activates glutathione (GSH). These stressors inhibit the expression of oxidative stress suppressive factors. ROS, GSSG, LPO & H_2O_2, all related to oxidative stress, were decreased by GSH. (Akter et al., 2021).

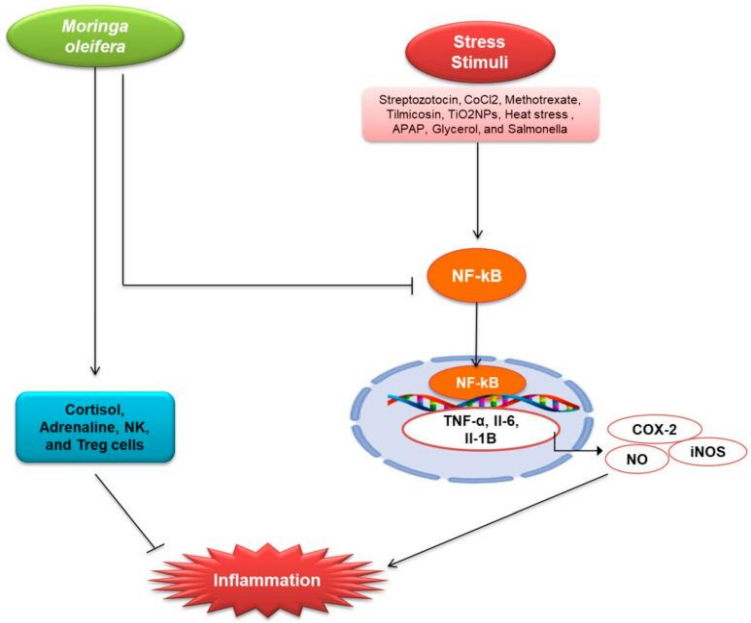

Figure 8: *Inflammation-fighting properties of the medicinal plant M.oleifera for the kidneys (Source:Akter et al., 2021).*

Figure 8 describes how *M.oleifera* protects the kidneys against inflammation is. The appearance of C-reactive protein (CRP), which activates NF-kB in the cytosol, is co-related to stress factors. When NF-kB enters the nucleus TNF-, Il-1B, iNOS, Il-6, & COX-2 are all activated & binds to DNA. These elements are linked with the development of inflammation. The presence of inducible nitric oxide synthase (iNOS) activates NO even more. NO is considered as a pro-inflammatory mediator which causes inflammation.

Again, in the above figure 8, it is seen that *M.oleifera* suppressed the expression of CRP & NF-kB in the cytosol. It also boosted cortisol, Regulatory T cells (Treg cells), natural killer cell (NK), & adrenaline which helped in reducing inflammation. Anti-inflammatory hormones Cortisol & Adrenaline Both NK cells & Treg cells are anti-inflammatory regulators (Akter et al., 2021).

3.2.1 Role *of M.oleifera* in reducing kidney disease by preventing diabetes

Diabetes is a chronic hyperglycemic condition that affects the blood glucose system and is characterized by pancreatic beta cells that are unable to produce enough or any insulin, leading to microvascular (retinopathy, neuropathy & nephropathy) and macrovascular (cardiovascular) complications. Oxidative stress induced by untreated hyperglycemia is linked to the development of diabetes and can result in serious consequences. Diabetes patients have elevated levels of leukocyte activation, inflammatory cytokines & increased tissue fibrosis. Oxidative stress, which is brought on by an accumulation of ROS, is linked to greater harm to -cells and biomolecules. Extremely severe outcomes like nephropathy, retinopathy, neuropathy, ketoacidosis, malignancy, rheumatoid arthritis, and coronary heart disease have been linked to an overproduction of ROS (Omodanisi et al., 2017).

M.oleifera offers a wide span of medicinal benefits and a high antioxidant content. It is found to have protective effect against inflammatory cytokines & oxidative status in the kidneys of diabetic rats. *M.oleifera* was previously found to have the capability to prevent the onset and consequences of kidney injury induced by diabetic. This plant is useful in modern medicine due to its anti-diabetic, analgesic, antispasmodic, antihypertensive, diuretic, cholesterol-lowering, antibacterial characteristics and antioxidant (Omodanisi et al., 2017).

There have been several reports of studies using animal models to examine the association between antioxidant properties in vitro and in vivo systems. Studies conducted on animals revealed that the aqueous extraction from the leaf of *M.oleifera* has antioxidant capacity of increasing antioxidant status & reducing lipid peroxidation in a dose-dependent manner, whereas tests conducted in vitro revealed high antioxidant capacities and protective effects against ROS. *M.oleifera* was found to have anti-diabetic and hypotensive effects in albino rats, according to Edoga and colleagues (2017). This is because the plant acted as a hypoglycemic agent by lowering blood glucose levels and preventing further cellular damage (Omodanisi et al., 2017).

In figure 9 it is shown how *M.oleifera* can prevent diabetes.

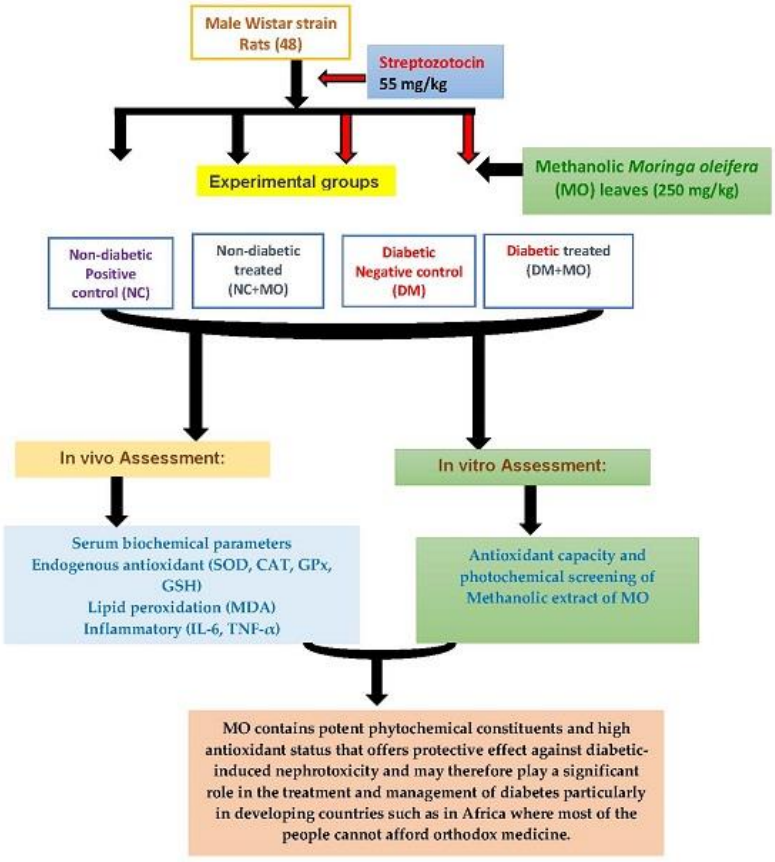

Figure 9: *Mechanism of action of M.oleifera in diabetic induced rat*

(Source:Omodanisi et al., 2017).

In figure 9, it is shown that 48 male wistar rats were induced with streptozotocin which is an antibiotic that is used experimentally to produce a model of type 1 diabetes Mellitus. 48 rats were divided into 4 groups. Among these 4 groups, 2 groups were treated with methanolic extracts of *M.oleifera* (250mg/kg). It is found that the groups treated with *M.oleifera* have shown positive effect against diabetic induces nephrotoxicity because of the antioxidant properties of *M.oleifera* (Omodanisi et al., 2017).

By giving free radicals an electron to stabilize them, antioxidants assist the biological system in self-defense, cleaning up and repairing damage caused by free radicals. The best and safest sources

of antioxidants are phytochemicals including carotenoids, flavonoids, glutathione, vitamins, carotene, tocopherols, flavonols, and polyphenols. It has been demonstrated that MO possesses high amounts of each of these antioxidant functions. The increased antioxidant intake each day can reduce the harm done by free radicals. It's interesting to note that MO's high phenolic content can prevent or reduce the oxidation of other molecules by snatching up free radicals and limiting the production of inflammatory cytokines. As *M.oleifera* is very rich in antioxidants is it believed to have antidiabetic properties which can prevent diabetes-induced nephrotoxicity (Omodanisi et al., 2017).

3.2.2 Role of *M.oleifera* in kidney disease by reducing high blood pressure

After diabetes, high blood pressure or hypertension is the second most common factor of kidney failure (Stompór & Perkowska-Ptasińska, 2020).

M.oleifera leaf extract reduces high blood pressure by reducing oxidative stress and vascular dysfunction. Rats given L-NAME exhibited dramatically elevated heart rate and blood pressure. In a dose-dependent manner, concurrent oral treatment with *M.oleifera* leaf extract (MOE) (30 and 60 mg/kg/day) may reduce tachycardia & elevated blood pressure. In isolated mesenteric artery beds, MOE lessened the impairment of acetylcholine-induced relaxation and the hyperreactivity of adrenergic-mediated contraction in response to periarterial nerve stimulation and phenylephrine. Furthermore, MOE showed antioxidant benefits in the hypertensive rats as shown by the inhibition of vascular O2•- generation, reduction of plasma and thoracic aorta MDA levels, and enhancement of SOD and CAT antioxidant activities. Additionally, in isolated methoxamine-precontracted arterial beds from L-NAME hypertensive rats, MOE (0.001-0.3 mg) induced a dose-dependent relaxation that was reversed by endothelium denudation (Direk et al., 2019).

3.2.3 Anti-inflammatory properties of *M.oleifera* in preventing kidney disease

Kidney is the body's filter. The kidneys develop acute nephritis when they become rapidly inflamed. There are many causes of acute nephritis, and if it goes untreated, it might eventually result in renal failure (Macon, 2019). Renal tubules can be damaged by acute or chronic diseases such as ischemia, inflammation or toxins, which can lead to kidney fibrosis & a lower GFR. Inflammation causes the glomeruli to scar over time, which can occasionally result in chronic kidney disease (CKD) or end-stage renal disease (ESRD).

These diseases can also impact the blood vessels that supply the kidneys. There is a correlation between the generation of cytokines levels & damage to the kidneys, which in turn extends the acute phase of renal disease. In addition, chronic inflammation is considered to be a comorbid disease associated with CKD diosmin. Active compounds in a variety of plants, including hesperidin, withaferin, fucoidan, & thymoquinone, amongst others, have been shown to have an anti-inflammatory effect. *M.oleifera* is shown to have anti-inflammatory properties, & such properties have been described here. It has been found that *M.oleifera* demonstrates highly active pro-inflammatory properties (Kusmiyati & Keman, 2018).

M.oleifera may lessen the expression of cytokines that promote inflammation in atopic dermatitis-prone BALB/c mice model. In experimental models, polar and non-polar extracts could both reduce inflammatory reactions. Polyphenols, phenolic acids, flavonoids, glucosinolates, tannins, saponins, oxalates, and phytates are all present in *M.oleifera*. The most prevalent polyphenol is flavonoids, which are mostly present in vegetables, fruits, leaves, roots, stems, etc. Flavonoids include the flavanol (catechin) group as well as flavones, flavanones, isoflavones, and anthocyanins. Its effects are linked to anti-inflammatory activity, according to some research (Kusmiyati & Keman, 2018).

In male Wister rats that had been stimulated with Streptozotocin (STZ), the methanolic extract of *M.oleifera* was able to diminish inflammation by lowering the levels of monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor, & Interleukin 6 (IL-6), which is a significant chemokine. Manufacture of inflammatory markers is reduced by *M.oleifera* & the representation of nitric oxide synthase & cyclooxygenase-2 by lowering the phosphorylation of mitogen-activated protein kinase (MAPK) pathway, as discovered the efficiency of an ethanolic extract of *M.oleifera* on metformin induced mice. In rats that have had $CoCl_2$ administered intraperitoneally, the ethanolic extract of *M.oleifera* hampers the production of inflammatory cytokines, including NO, which plays a role in the pathophysiology of inflammation.

Leaf extract from *M.oleifera* suppresses the manufacturing of inflammatory cytokine & controls the swelling by reducing the activity of the NF-kB gene (Akter et al., 2021). Extracts of *M.oleifera* were found to diminish inflammation in rats that had Tilmicosin-induced inflammation. This was again a crucial finding. Through the process of lowering the Kidney injury molecule-1 (KIM-1) in 4 Titanium dioxide particles (TiO_2NPs) incited male albino rats, the leaf extract of *M.oleifera* provides protection against interstitial renal inflammation with fibrosis. Research has shown that the extract of *M.oleifera* stimulates the manufacture of anti-inflammatory cytokines & promotes the release of leptin & cortisol, as well as raises the number of regulatory T cells & natural killer cells. The administration of *M.oleifera* therapy decreased the uttering of Tissue inhibitors of metalloproteinases (TIMP-1), KIM-1 & Tumor necrosis factor (TNF) in male Sprague Dawley rats that had been given ML. TNF is an inflammatory cytokine which incites Interleukin-1 (IL-1) & IL-6, both of which are decreased by *M.oleifera* in seabream, & activated Transforming growth factor (TGF) elicits anti-inflammatory activities. In mice that had been treated with Automatic positive airway pressure (APAP), which is known to promote AKI. *M.oleifera* was found to lower

the levels of inflammatory cytokines. Fermented extract from leaves also has the effect of lowering nuclear factor erythroid 2–related factor 2 (NRF2) levels in rats that have been stimulated by Salmonella (Akter et al., 2021).

When compared to curcumin, the phytochemicals found in moringa seed have the ability to inhibit the generation of nitric oxide, as well as gene expression of LPS-incitable iNOS & interleukins 1 & 6. It has been demonstrated that flavonoids are efficient inhibitors of the actions of nitric oxide synthase type 2. In addition, flavonoids inhibit the activity of protein tyrosine kinase, which is also involved in the expression of NOS-2 at the molecular level. Flower extract has the potential to stimulate the production of proteins that are involved in inflammation, including toll-like receptors. Quercetin & kaempferol, which are found in the flowers, have the ability to incite the signal transducer & activator of transcription 1 pathway as well as the NF-B pathway. Research has shown that the blooms of *M.oleifera* contain 80 percent hydroethanolic, which is a dominant anti-inflammatory drug in NF-kB signaling pathway. Scientists have come across phenolic glycosides repress inducible iNOS expression & NO production in mouse macrophage cells, as well as COX-2 & iNOS proteins. Because both the seeds & the flowers of the moringa plant contain a large number of bioactive components, moringa extracts gradually bring the inflammatory mediators under control. Each of these chemicals is responsible for its own unique set of actions (Akter et al., 2021).

Prostanoid biosynthesis inhibition, histamine release inhibition, phosphodiesterase inhibition, protein kinase inhibition, and transcriptase activation inhibition are key anti-inflammatory mechanisms. 5-desmethylnobiletin, 3,5,6,7,8,38,48-heptamethoxy flavone (HMF), sinensetin, 5-hydroxy-3,6,7,8,38,48-hexamethoxyflavone and nobiletin, were the most effective flavonoid inhibitors of TNF-. TNF- was suppressed at the transcriptional level. TNF- was also moderately

suppressed by a number of hydroxylated flavones, including apigenin, rhamnetin, quercetin, tamarixetin and kaempferol. Clinicopathological analysis of the organs of rats given oral *M.oleifera* doses at 400, 800 &1600 mg/kg revealed no major abnormalities, leading researchers to draw the conclusion that the plant is generally safe for use as food and medicine (Kusmiyati & Keman, 2018).

As the kidney plays a significant role in homeostasis, and inflammation causes significant harm to renal function. Moringa shows great anti-inflammatory response, the methanolic and ethanolic extract of moringa has proven to be very effective in reducing inflammation.

3.2.4 Anti-antioxidant properties of *M.oleifera* in preventing kidney disease

Oxidative stress is a phenomenon brought on by an imparity between a biological system's capacity to detoxify these reactive byproducts and the creation and buildup of oxygen reactive species (ROS) in cells and tissues. Although ROS is a by-product of oxygen metabolism and can play a variety of physiological roles such as environmental stressors like UV, including cell signaling, pollutants, heavy metals and ionizing radiation, as well as xenobiotics like antiblastic drugs all contribute to significantly increased ROS production, which creates the imparity that results in cell and tissue damage (Pizzino et al., 2017).

Free radicals are produced by enzymatic processes such as those in the phagocytosis, respiratory chain, the cytochrome P-450 system, and prostaglandin production. Both ionizing processes and nonenzymatic reactions involving oxygen and organic molecules can result in the formation of free radicals. It is a common symptom of CKD and has evolved into a diagnostic indicator because of its prevalence. Superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST) & catalase's (CAT) activity ware evaluated as

indicators of antioxidant defenses. The production of superoxide radical (O2(•-)) was measured as an indicator of reactive oxygen species production, protein carbonyl levels, & lipid peroxidation (TBARS)were quant (Alavez, 2019).

In a number of different research projects it has been shown that *M.oleifera* possess antioxidative characteristics, which can either prevent or mitigate cellular damage. The methanolic extract of leaves contain tannins, flavonoids, steroids, terpenoids, alkaloids, and cardiac glycosides. In minor amounts, phenolic chemicals, flavonoids, and alkaloids have been discovered in earlier research. In addition, there were ascorbic acids, sterols, isoquercetin glucoside, carotenes, and kaempferitrin. Total flavonol, flavonoid, and phenolic concentrations in *M.oleifera* leaf extract were determined to be 12.12 mg/g of QE, 40.5 mg/g of QE, and 120 mg/g of GAE respectively. Leaves include quercetin (89.8 mg/100 g fw), kaempferol (36.3 mg/100 g fw), and isorhamnetin (2.9 mg/100 g fw) for a total flavonoid content of 129 mg/100 gfw (Kusmiyati & Keman, 2018).

Methanol extract of *M.oleifera* was found to minimize the oxidative stress that was caused in male rats by STZ. This was accomplished by lowering the generation of ROS, LDL, & MDA, CHOL, all of which are known to raise the risk of CKD. In ischemia-induced Wistar rats, the administration of methanol extract reduced the production of oxidative stress-causing molecules such as MDA, NO, H_2O_2, GPx, AOPP, & GST. Another study demonstrated that patients with CKD had lower levels of blood urea nitrogen (BUN) and creatinine after using metabolic extract, and these patients also had higher levels of total protein. Ethanolic extract of *M.oleifera* reduces LDL cholesterol, which in turn decreases oxidative stress & atherosclerosis in patients with CKD. It has been shown that ethanolic extract from *M.oleifera* can reduce the oxidative stress that 8-OHdG causes to DNA & the subsequent promotion of cancer. Ethanol extracts speed up the whole process of creatinine clearance, which results in a lower overall amount of creatinine in the plasma.

After treatment with an ethanolic extract from *M.oleifera* to nickel-induced Wistar rats, both sodium & potassium levels in the plasma were shown to be elevated. In ML-induced male Sprague Dawley rats, the administration of ethanolic extract resulted in a detoxification of the plasma by lowering levels of bilirubin (direct & indirect), urea, & other biomarkers. Leaf extract was administered at doses of 300 & 400 mg/kg body weight, respectively, & both nuclear factor erythroid2 (Nrf2) & Heme oxygenase-1(HO-1) expression was found to be increased, which inhibits the progression of inflammation (Luo et al., 2018). The amount of total thiol TiO2NPs generated in male albino rats was up-regulated as a result of the administration of leaf extracts, which is significant for the provision of antioxidant protection. The oxidative stress-producing intermediary in sodium fluoride induced gentamicin-induced rabbit, Nile tilapia, & APAP-treated mice were all inhibited by leaf extract of *M.oleifera* (Akter et al., 2021).

Various cultivars of *M.oleifera* have different concentrations of flavonoid molecules in their leaves. Gallic acid (49.074.53), chlorogenic acid (286.13), luteolin (44.562.03), rutin (603.3513.48), quercetin (46.180.6), kaempferol (46.432.14), and apigenin were identified as the polyphenolic components present in the 80% methanolic extract of *M.oleifera* leaves collected from San Pedro (24.412.16). Gallic acid (43.28, chlorogenic acid (479.53), luteolin (94.277.6), rutin (845.2518.83), quercetin (49.896.98), kaempferol (67.367.86), and apigenin were among the polyphenolic chemicals found in Lombardia (g/dry matter) (8.740.95). *M.oleifera* leaves extracted with 100% ethanol using the shaker and reflux extracting procedures had total flavonoid levels (CE g/100 g DW) of 5.33 and 4.19, respectively. *M.oleifera* leaves extracted with 80% ethanol using the shaker and reflux extraction procedures had total flavonoid levels of 6.21 and 5.31, respectively. When compared to absolute ethanol and absolute methanol, 80% ethanol and 80% methanol extractions had stronger antioxidant activity. The maximum concentration of flavonoids

was obtained from a subcritical ethanol extraction using 70% ethanol at 126.6 °C for 2.05 hours. The extract displayed significant antioxidant and free radical scavenging properties, as determined by the FRAP and DPPH experiment. The mature stage of the leaves may have an impact on the constituents (Kusmiyati & Keman, 2018).

In iodide-injected rabbits, an alcoholic extract of *M.oleifera* lowered oxidative stress by reducing the amount of lipid peroxidation & reactive oxygen species. In addition to this, the fermented leaf extract of *M.oleifera* raises the levels of antioxidant activity in mice that have been caused by bacteria. The manifestation of MDA was reduced, which indicates that the free radical increased production was lowered in both Tilmicosin & Hg induced rats. *M.oleifera* extract was responsible for this reduction. According to the findings, the level of superoxide dismutase was elevated in rats after receiving therapy with *M.oleifera*. All live cells contain the enzyme superoxide dismutase (SOD). An enzyme is a substance that has the potential to quicken chemical processes occurring in the biological system. In cells, superoxide dismutase aids in the degradation of potentially damaging oxygen molecules. Tissue injury might be avoided in this way. Superoxide dismutase (SOD) is an essential antioxidant defense mechanism in the body against oxidative stress. The enzyme is an effective treatment for illnesses brought on by reactive oxygen species (Younus, 2018).

In rats with Beryllium-induced nephropathy, hydroalcoholic root extract increased blood sugar levels, antioxidant enzyme work rate, & G-6-phase work rate, all of those protect the kidney from the effects of nephropathy. In lead-treated rats, the administration of seed powder decreased the levels of free radical species, TPCC, & metal content, & it boosted ALAD activity. Seed powder from *M.oleifera* significantly improved antioxidant function in rats that had been treated with

arsenic. This improvement included Aminolevulinate dehydratase (ALAD) & Glutathione (GSH) (Akter et al., 2021).

There are recognized kidney toxicity effects of lead. Through altering the kinetics and distribution of lead in the blood, bones, and internal organs, the ALAD gene polymorphism is a significant factor influencing human susceptibility to lead toxicity (Siha et al., 2019).

Glutathione (GSH) needs to be present in sufficient quantities in order for the kidneys to continue operating normally. High rates of aerobic metabolism, especially in the proximal tubules, contribute to this. High quantities of reactive electrophiles and oxidants may also be present in the kidneys (Lawrence, 2005).

Due to the extracts' lack of toxicity, administration of doses up to 100 mg/kg BW could still be tolerated. Studies on antioxidant activity in vivo and in vitro revealed that frequent consumption of the plant's leaves through diet could shield patients from oxidative damage. The extracts have the ability to neutralize free radicals and have a protective impact against oxidants, which are responsible for cellular damage. An in vitro investigation revealed that the leaves extract might also boost the activity of liver enzymes linked to fighting ROS and protect against oxidative harm brought on by diabetes (29). When administered to Wistar rats exposed to cement dust, ethanol extract (95%) at a concentration of 400 mg/kg BW demonstrated its antioxidant potential (Kusmiyati & Keman, 2018).

3.2.5 Anti-fibrotic properties of *M.oleifera* in preventing kidney disease

The last stage of chronic kidney disease is renal fibrosis, which is characterized by tubulointerstitial fibrosis and glomerulosclerosis (CKD). One of the main issues in nephrology,

the progression of CKD, shows that patients eventually develop end-stage renal disease (ESRD) and need renal replacement therapies like dialysis and transplantation (Cho, 2010).

The TGF-1-SMAD pathway & hypoxia is recognized to be the primary regulators of epithelial-mesenchymal transition. The epithelial-mesenchymal transition is the primary mechanism through which kidney fibrosis occurs. *M.oleifera* extract is able to inhibit the uttering of fibronectin, type I collagen & type I collagenPAI-1 in rat kidney fibroblast cells that have been stimulated by TGF. Both in vivo and in vitro tests on kidney protection showed promise for *M.oleifera* seed extract. In order to preserve the kidneys, Nrf2/HO-1 and GSK-3 were activated. In a study, GSK-3 was identified as a potential anti-renal fibrosis factor (Wen et al., 2021).

In addition, the root extract of the moringa plant was able to selectively inhibit the phosphorylation of Extracellular signal-regulated kinase (ERK) & SMAD4 that was triggered by TGF-. Based on these findings, it appears that the antifibrotic activity of the moringa root extract in rat kidney fibroblast cells may be responsible for the reduction of renal fibrosis by the moringa root extract. Rats that were given *M.oleifera* seed extract orally showed a reduction in the amount of hepatic fibrosis caused by CCl4. Congenital hepatic fibrosis (CHF) and autosomal recessive polycystic kidney disease (ARPKD) are fibrocystic diseases that primarily affect the kidney. Fusiform dilatations of the renal collecting duct and ductal plate malformation of the liver are its defining features. It occurs roughly once in 20,000 live births. The PKHD1 gene, which produces fibrocystin/polycystin, has mutations that account for the bulk of cases (Srinath & Shneider, 2012).

3.2.6 Effect of *M.oleifera* in Creatinine level

Elevated creatinine level in the blood is a warning sign of renal dysfunction, a condition that is linked to an increased risk of death in patients all over the world. It is generally agreed upon that

a serum creatinine level of 1.5 mg/dl should serve as a benchmark for the upper limit of the 95th percentile of normal values. Because of this, renal insufficiency is often diagnosed when the serum creatinine level is more than or equal to 1.5 mg/dl. Patients whose blood creatinine levels are 1.2 mg/dl or above have been demonstrated to have experienced a loss of 50 percent of their renal function & are recommended to undergo prompt therapy. Elevated amount of serum creatinine is often associated with complications following cardiac surgery, patients with atherosclerotic disease, diabetes mellitus, & several other reported instances. This is in addition to the negative effects that high amount of serum creatinine has on the renal system. In fact, elevated serum creatinine levels are related to an enhanced danger of major cerebrovascular disorders in individuals who have normotensive blood pressure as well as individuals who have hypertensive blood pressure (Sahoo et al., 2016).

Studies that are related to this have shown that higher levels of serum creatinine are a common element in all-cause mortality as well as the other causes of death. In order to avoid the issues that are brought on by elevated serum amount of creatinine, it is urgently necessary to find a method that is both effective & rapid in reducing the levels of serum creatinine down. The majority of patients with excessive serum creatinine amount have been treated with dialysis, changes in food & lifestyle, & frequently with some drugs from Chinese medicine that are very dimly understood. Ketosteril is frequently used as a nondialysis method for the treatment of high serum creatinine; nevertheless, it is limited in its effectiveness due to side effects such as hypercalcemia, which leads to cardiac arrhythmia. In light of these reports, the administration of compounds derived from natural sources such as *M.oleifera,* which have an inhibitory action on increased levels of serum creatinine & do not have any side effects that are known, can open up a new vista for the treatment of the clinical manifestations that have been discussed. The use of substances with anticreatinine

properties can lessen the degree of the damage & prevent, at least in some ways, the development of additional difficulties (Sahoo et al., 2016).

Blood samples with high serum creatinine concentrations (2 mg/dl and above) were treated with *M.oleifera* leaf extract to address this problem. The initial partial purification of the crude extract was followed by a final full purification. As previously mentioned, samples with high serum creatinine levels were treated using all of these proteinaceous fractions. While serum samples with high serum creatinine levels treated with crude extract and partially purified protein fractions showed a decrease in serum creatinine levels of about 20% over the course of 24 hours, samples treated with purified protein fraction showed a reduction in serum creatinine levels of 50% (Sahoo et al., 2016).

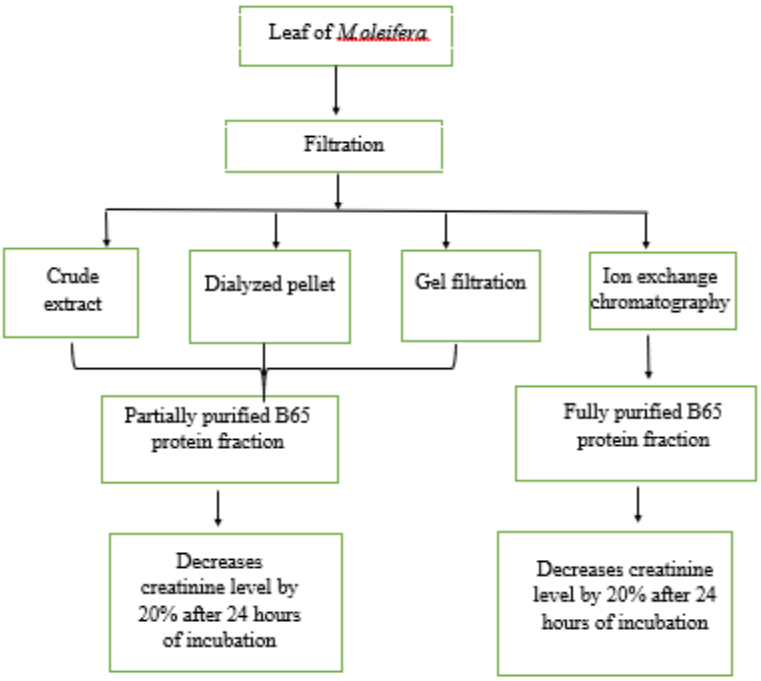

Figure 10: *Different processes of filtering protein components in M.oleifera and checking their effect on blood creatinine level.*

In Figure 10, the leaf of *M.oleifera* was filtered in different techniques and then the prominent protein fraction was selected. Later, it was tested on blood samples where serum creatinine level was high. It was seen that after administrating the blood sample with this protein serum creatinine level decreased by 50%. The whole mechanism has been explained in detail below.

In November of 2011, mature, fresh *M.oleifera* leaves were taken from Madgaon city in Goa, India. After being stored in the shade for two weeks, the leaves were allowed to dry. After the leaves had completely dried out, they were pulverized using a mortar & a pestle to get the powder ready for extraction. After everything was done, the powder was sieved & put away in a dry storage in a dark spot so that it could be used in other experiments. The method that Jabeen et al. used to prepare the *M.oleifera* leaf extract was tweaked ever-so-slightly so that it could accommodate the little change that was made. The powder from one gram of *M.oleifera* leaves was suspended in six milliliters of extraction buffer (10 mM potassium phosphate, pH 7.0). As a protease inhibitor, ten millimolar phenylmethylsulfonyl fluoride (PMSF) was added to the mixture, & then the whole thing was vortexed for thirty seconds. This combination was spun in a centrifuge at a speed of 10,000 rpm & 4 degrees Celsius for twenty minutes. The resultant extract was filtered before being kept at a temperature of 4 degrees (Sahoo et al., 2016).

It was determined that the crude extract of *M.oleifera* leaf, after it had been prepared, had a total protein concentration of 3 mg/ml. The ammonium sulfate precipitate was added to this crude extract, & it was processed. It was standardized in order to ensure the maximum possible separation of elements at a saturation level of fifty percent. Both the supernatant & the resuspended pellet were subjected to separate overnight dialyzes against the extraction buffer before being kept as the fractions S (supernatant) & P (pellet), respectively, for subsequent purification. Gel filtration

chromatography with a Superdex 200 prep grade column from GE Healthcare LifeSciences was used to differentiate S & P into additional fractions in a manner that was separate from one another (Uppsala, Sweden).

When checking the purity of the samples, it was found that the predominant proteins in the pellet fractions was a specific protein called B65, which had a molecular weight of approximately 65 kDa. Despite this, the purification that occurred following the gel filtration phase was just partial & not complete. Therefore, the fractions obtained from gel filtration were put through a second round of purification using ion-exchange chromatography with a GE Healthcare LifeSciences HiTrap Q sepharose prepacked column as the stationary phase (Uppsala, Sweden). The final fractions revealed that the protein B65 had been virtually entirely purified (figure 11) (Sahoo et al., 2016).

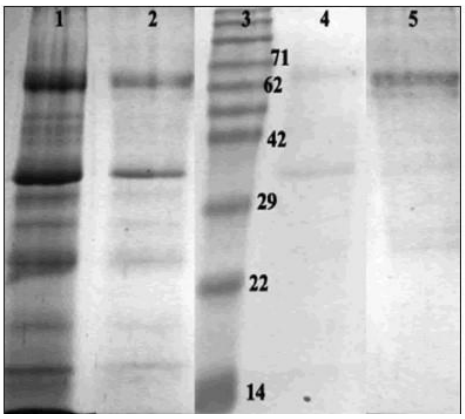

Figure 11*: SDS-PAGE (Sodium dodecyl-sulfate polyacrylamide gel electrophoresis)profile of purification of crude extract of Moringa oleifera Lam (Source:Sahoo et al., 2016).*

In figure 11, the lanes denote the amount of protein present in moringa sample after various filtration process. Lane 1: Crude extract of *M.oleifera*, Lane 2: Pellet of Ammonium sulphate,

Lane 3: NEX-GEN-PinkADD prestained protein ladder 10-175 kDa, Lane 4: Purified fraction of protein after gel filtration, Lane 5: Purified fraction of protein after ion exchange purification. The figures beside lane 3 indicates the molecular weight of proteins in kDa (Sahoo et al., 2016).

Blood sample was taken with a high serum creatinine (at least 2 mg/DL). After being treated with (1) partially purified protein fragment P65, (2) ammonium sulphate precipitating fraction P, (3) crude *M.oleifera* leaf extract, & (4) purified protein fragment P65, a blood serum sample of fifty microliters was evaluated. In each & every one of the tests, the sample of blood serum was given an addition of fifty microliters of the extract or the purified extract. In order to achieve a consistent distribution, the total amount of protein measured in milligrams (g) was maintained throughout all of the samples that were processed. After being kept at room temperature for 1, 2, 4, & 24 hours, the sample extract mixes were then tested for blood creatinine levels at each of the aforementioned time intervals. In every experiment, a control consisting solely of the increased serum creatinine specimen was simultaneously inoculated under the constant conditions as the other samples (Sahoo et al., 2022).

The AutoZyme creatinine test kit used a colorimetric detection approach in order to determine the amount of creatinine present in the serum. This was done using the initial rate method (Accurex Biomedical Pvt. Ltd.). The sodium hydroxide diluents & sodium picrate were the two most important testing agents in the kit that was utilized for the distinguishing process. The instructions that came with the kit were followed in order to arrive at a reasonable estimate of the level of creatinine present. All measurements were taken in relation to a reference solution that was provided with the kit, which contained creatinine level of 0.1 mg/DL. According to the following formula, the sum of creatinine that was found in every sample was determined:

Serum creatinine (mg/dl) = $(\Delta_{spec}/\Delta_{std}) \times 2$, where Δ_{spec} is the average difference in absorbance per minute for the sample, & Δ_{std} is the same value for the standard (Sahoo et al., 2016).

The presence of large amounts of interference & fluid from pigments makes it difficult to extract anything from plant matter. This applies to all types of plant matter. As a result, it was distinct to extract the material from the resuspended & dry mass of leaves by employing an awash method of extraction. When the dry mass extraction dealt with the elevated fluid volume & boosted the yield of protein, the awash extraction avoided disruption from the pigment component. We concentrated on identifying the various protein components found in the leaf of the *M.oleifera* plant because it had been stated in a previous study that the leaf is an abundant origin of proteins. Such as, Arginine, Lysine, Histidine, Tryptophan, Methionine, Phenylalanine, Threonine, Leucine, Valine & Isoleucine (Dhakar, 2011). In light of this, prior to conducting the investigation, it was decided to conduct a check on the pursuit of the *M.oleifera* leaf proteins.

Despite the fact that the raw extract of the *M.oleifera* leaf contained a higher variety of ammonium sulphate fragmentation, proteins, was able to segregate these proteins into either S or P fragments. It was chosen to evaluate the activities of the proteins found in the P fraction when it was discovered that the amount of protein found in the P fragment was more than that of the S fraction (figure 11). Because the purification of P using gel filtration produced unsatisfactory results, these fractions had to be repurified utilizing a linear gradient of potassium chloride on a HiTrap Q Sepharose column (figure 11). The phase involving ion exchange resulted in the production of purified portions of a protein,that was the predominant component of the extract. P65 was chosen as the name for this protein because it was found to be concentrated in the region of the 12.5 percent SDS-PAGE gel that corresponds to the 65 kDa band on the Nex-Gen Protein Ladder (Sahoo et al., 2013).

The inceptive treatment of a blood serum sample that had elevated levels of creatinine (3.3 mg/DL) with the raw extract & the ammonium sulphate purification fragment P demonstrated that there was a slight decrease in the number of serum creatinine of the elevated creatinine sample over a period of time ranging from 1 to 4 hours. The extent of the drop was up to a maximum of 0.2 mg/DL for the first four hours after administration. Because of this, we were able to draw the conclusion that the component that was accountable for the anticreatinine pursuit of the *M.oleifera* leaf extract should have also been purified in the ammonium sulphate fragment P.

As a consequence of this, more attempts were made to purify the fraction P. When patients with high creatinine levels were given P65's pure protein fractions as medication, a trend toward decreased creatinine levels was seen, albeit in a significant manner. The anticreatinine outcome of the raw extract, the dialyzed rediffused pellet P, & the partially refined fraction are more or less comparable. This is obvious from the comparison results obtained after 24 hours of incubation as well (figure 6), which show that the anticreatinine outcome of the raw extract. On the other hand, treatment with the pure P65 specimen for the exact amount of time (24 hours) resulted in a sizeable reduction (by fifty percent) in the amount of serum creatinine that was present. This experiment was carried out multiple times with different samples, all of which had creatinine levels that were more than 2 mg/dl, & identical results were obtained each time (Sahoo et al., 2016).

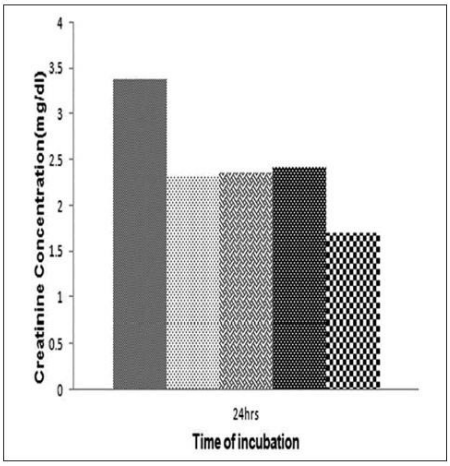

Figure 12*: Effect of treatment with different levels of purified extract on high serum creatinine sample (Source:Sahoo et al., 2016).*

In figure 12, it is shown that: Effect of treatment of high creatinine sample with crude extract, dialyzed pellet, purified gel filtration fraction and IEX(Ion exchange chromatography) purified fraction after 24 h of incubation (Sahoo et al., 2016). From figure 7 it is very clear that protein fractions of moringa that are purified by IEX is most effective in reducing blood creatinine level.

Chapter 4

M.oleifera in preventing kidney disease

Because natural sources tend to have fewer adverse effects than synthetic sources, researchers are focusing their efforts on the development of medications derived from natural sources rather than developing synthetic drugs. Researchers from Nigeria demonstrated how *M.oleifera* is an herb that is good for the body & does not cause any harm to the kidneys. According to the findings of another study, greater dosages of *M.oleifera* caused toxicity in rats, but a moderate level dose of *M.oleifera* was shown to be safe. It has been demonstrated that treatment with *M.oleifera* can improve diabetic nephropathy in alloxan incited rats. Acetaminophen is harmful to the liver & kidneys, however a treatment with *M.oleifera* at a dosage of 500 mg/KG is effective in reversing this damage. Saleh et al. found that *M.oleifera* prevented necrosis & expansion of renal tubules in Cd-induced rats. Based on these findings, they hypothesized that *M.oleifera* could be utilized as a medicinal plant. Extracts of the leaves of the *M.oleifera* plant were able to minimize oxidative stress as well as damage to the kidneys & liver. According to the findings of a study that used a randomized placebo-controlled design, taking capsules made from the leaf of the *M.oleifera* plant may help reduce both high blood sugar & high blood pressure. Additionally, aqueous extracts of *M.oleifera* have been shown to lower the toxicity of metals & have shown protective properties in Saccharomyces cerevisiae (Akter et al., 2021).

Table1: Summary of Reno-protective effect of *M.oleifera* in Kidney disease.

Reference	Journal name	Experimental model	Sample size	Dose of *M.oleifera*	P value
(Karthivashan et al.,2016)	PeerJ (Malaysia)	Male Balb/c mice	30	200 mg/kg of bw.	APAP-induced Nephrotoxicity (decreased) <0.05, Serum kidney biomarkers (increased) <0.05, Inflammatory markers (decreased) <0.05
(Oguntibeju et al.,2020)	South African Journal of Botany (South Africa, Namibia)	Male wistar rats	48	250 mg/kg	Serum creatinine, (decreased) Albumin and Bilirubin concentrations (increased) <0.05 Inflammatory cytokines (decreased) <0.05
(Adedapo et al.,2020)	Scientific African (Nigeria, South Africa)	Wistar rats	35	50 mg/kg, 100 mg/kg	MPO, Creatinine, NO, BUN (decreased) <0.05
(Karadi et al.,2009)	Pharmaceutical Biology (India)	Wistar albino mice	42	200 mg/kg	Urinary excretion, Creatinine, Blood urea nitrogen, levels of oxalate, calcium and phosphate (decreased) <0.001,
(Hegazy et al.,2020)	European Journal of Anatomy (Egypt)	Male albino mice	28	400 mg/kg,	Kidney weight (increased) <0.001. Serum creatinine, BUN (decreased) <0.001
(Oyagbemi et al.,2021)	Journal of Pharmacognosy	Male wistar rats	35	50 mg/kg, 100 mg/kg	Serum creatinine, total protein level, blood urea nitrogen

	& Natural Products (Nigeria)				(decreased) <0.05
(Akinlolu et al.,2014)	International Journal of Morphology (Nigeria)	Male wistar rats	25	250 mg/kg, 500 mg/kg, 750mg/kg	Alanine and Aspartate Transaminases (increased) <0.05, Serum urea Concentration (no significant differece) <0.05
(Mohamed et al.,2020)	Asia pacific journal of tropical biomedicine (Egypt)	New Zealand White rabbits	36	400 mg/kg	Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, creatinine (decreased) <0.01 Blood glucose level (increased) <0.01
(Aliyu et al.,2021)	SAGE journals (Malaysia)	Female ICR-mice,	10	2000mg/kg	BW (Body weight) (increased) >0.05, Relative organ weight (No significant difference) >0.05, Urea(decreased) >0.05 Creatinine (increased) >0.05
(Ezejindu et al.,2014)	American Journal of Engineering Research (Nigeria)	Adult wistar rats	24	0.5 ml, 0.6 ml, 0.7 ml	Body weight, kidney weight (increased) <0.001
(Saleh et al.,2021)	IOP Conference Series: Earth and Environmental Science (Iraq)	Male rats	66	100 mg/kg, 200 mg/kg, 300mg/kg, 400mg/kg	Urea, Serum Creatinine (decreased) <0.05

(Paliwal et al.,2011)	Biology and Medicine (India)	Male mice	10	200 mg/kg, 400mg/kg	Increases the anti-oxidant level. Decreases glutathione, glutathione-S-transferase
(Purena et al.,2019)	Toxicology international (India)	Male wistar rats	42	200 mg/kg, 400mg/kg	Blood urea nitrogen, Creatinine (decreased) <0.05
(Sharma et al.,2012)	Asian Pacific Journal of Cancer Prevention (India)	Adult Swiss albino male mice	60	200 mg/kg, 400mg/kg	Kidney weight (decreased) <0.001
(Akinrinde et al.,2020)	African Health Sciences (Nigeria)	Wistar rats	42	200mg/kg, 400mg/kg	malondialdehyde (MDA), advanced oxidation protein products (AOPP), PC, BUN, Creatinine (decreased) <0.05
(Abou-Zeid et al., 2021)	Biomedicine & Pharmacotherapy (Egypt)	Sprague–Dawley male rats	50	800mg/kg	Inflammatory markers/biomarkers (decreased) <0.05
(Nafiu et al.,2019)	Biomedicine & Pharmacotherapy (Nigeria)	Male wistar rats	45	400mg/kg	Oxidative stress, Serum creatinine level (decreased) <0.05
(Adeyemi et al.,2014)	Journal of nutrition and metabolism (Nigeria)	Male wistar rats	30	5% 10% 15%	Plasma creatine,urea (decreased) <0.05 Electrolytes (increased) <0.05
(Soliman et al.,2020)	Biomedicine & Pharmacotherapy (Saudi Arabia, Egypt)	Male mice	32	300mg/kg	serum oxidative stress, urea, creatinine (decreased) <0.05
(Abou-Zeid et al.,2021)	Biomedicine & Pharmacotherapy (Egypt)	Male mice	60	400mg/kg 800mg/kg	Creatinine, total protein, Urea (decreased) <0.05
(Ahmed et al.,2020)	Animals (Egypt)	Nile Tilapia	264	1% w/w	Reduces oxidative stress

(Ouédraogo et al.,2013)	Experimental and Toxicologic Pathology (Belgium)	Rabbit	-	150mg/kg, 300mg/kg	lipid peroxidation (LPO) level (decreased) <0.01
(Velaga et al.,2014)	Journal of Environmental Pathology, Toxicology and Oncology (India, USA)	Male wistar rats	-	500mg/kg	ROS, LPP, TPCC (decreased) <0.05
(Altaee et al.,2021)	Journal of Pharmaceutical Research International (Iraq)	Male rabbits	21	250mg/kg	MDA (deceased) <0.001, GSH (increased) <0.001
(Omodanisi. ,2017)	Molecules (South Africa)	Adult male wistar rats	48	250mg/kg	inflammatory biomarkers (decreased) serum albumin, globulin, total protein (increased) <0.05

Table:1 summarizes the previous knowledge of some significant experiments conducted on different animal models. Sample size and dosage variation of MO in both controlled and treated animal models establish the potential protective role of MO in kidney disease treatment. The p-value <0.001 is a highly significant value, whereas p <0.05 is considered significant. MO is observed to be effective in lowering oxidative stress, high blood pressure, and diabetes by reducing harmful ROS, serum creatinine, albumin, bilirubin concentrations, LPP, TPCC, etc. Therefore, data from numerous sources establish the beneficial role of MO in kidney disease treatment.

This tree is absolutely necessary for ushering in a brand-new era of medicinal research because of the extensive phytochemical profile it possesses as well as the technical advancements that have been produced. A technique known as in vitro propagation offers novel insights into the process

of generating products that are more efficient, eco-friendly, & biodegradable through the application of production & mass multiplication methods. Even though it has been demonstrated that in vitro propagation procedures for *M.oleifera* are efficient, there will still be laps in the manufacturing of metabolites & in the specific metabolites that are found in the human body. The successful commercialization of essential plant products will be facilitated by the application of biotechnological methods. There is no shadow of a doubt that the implementation of biotechnological methods will pave the way for extensive research that will establish *M.oleifera* as one of the most important potential treatments for a wide range of health problems, including kidney illnesses (Akter et al., 2021).

Conclusion

The ability of the kidneys to operate properly decreases with age. The effectiveness of currently available medications for treating renal illnesses is hindered by the adverse effects of the drugs. As a result, natural substances that produce fewer adverse effects are currently under investigation. According to the research that was analyzed for this review of the literature, *M.oleifera* shows a number of pathological factors that are related to kidney disorders such as, oxidative stress, kidney fibrosis, blood-sugar level, high blood pressure & inflammation.

This research summarizes information on *M.oleifera*'s potential therapeutic effects against kidney illnesses. Additional research is required to validate the anti-kidney disease effects of the bioactive phytochemicals found in *M.oleifera*. The issues that have been brought up in this study will provide a future research avenue for determining how pharmacological therapies that are based on natural substances could affect renal disease. On the other hand, it would give information on how

medications based on *M.oleifera* could potentially act as a kidney restorative agent in the treatment of problems in the kidneys that are related with aging. In recent years, there has been lot of focus placed on the utilization of natural elements as a source of remedy due to the negative consequences that are caused by synthetic resources as well as the non-renewable nature of these resources. Medicine derived from *M.oleifera* would be a great preventative measure against a number of risk factors connected with renal disorders.

Limitation of the study

This study has some limitations. The experimental models were mostly rodents. Other species of animals should be tested with *Moringa oleifera* in order to strengthen its claim. Although the renoprotective properties of *M.oleifera* has been seen in various tests upon animal models, a specific dosage has not been suggested yet.

Future research plan

To this date *M.oleifera* has shown positive effects in protecting kidney from various risk factors such as diabetes, high blood pressure, inflammation, oxidative stress, fibrosis, serum creatinine. The studies mentioned above have only been done to experimental animal models. However, now it is the time to think for a clinical trial of *M.oleifera*. It is true that kidney disease is increasing in every country and one particular medicine is not available for it. Therefore, the inclusion of *M.oleifera* in the treatment of kidney disease will give mankind a boost.

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