WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Pharmacological Evaluation of Nephroprotective Activity of *Carica Papaya* in Wister Albino Rats

M Raviteja Reddy[1], A Srivani[2]

[1]Reg. No: 22031S0716], M. Pharm., [2]Assistant Professor
Jawaharlal Nehru Technological University Hyderabad

ABSTRACT

The study investigated the nephroprotective effects of chloroform carica papaya leaf extract (CCPLE) administered with gentamicin, a nephrotoxin, in a rat model. Rats received CCPLE at doses of 200 or 400 mg/kg orally, along with silymarin (200 mg/kg) for comparison, over an 8-day period. Results showed that CCPLE significantly improved renal function, evidenced by enhanced glomerular filtration rate (GFR) and increased serum protein, albumin, and globulin levels, while also reducing serum markers of kidney damage, such as creatinine, uric acid, urea, and blood urea nitrogen (BUN). Histopathological analysis of the kidneys supported these findings, revealing notable improvements in tissue structure and function in the CCPLE and silymarin-treated groups compared to untreated controls. The nephroprotective effects of CCPLE are likely attributed to its rich content of flavonoids, tannins, and other bioactive compounds that may help reduce oxidative stress and promote kidney health.

1.INTRODUCTION

1.1 Herbal Medicine

The study of plants and their potential to treat various illnesses is referred to as herbalism, herbology, or herbal medicine. Throughout history, medicinal plants have been recognized and utilized for their healing properties. For many years, they formed a crucial foundation for addressing a wide range of health issues. Herbal medicine utilizes various plant parts, including leaves, flowers, fruits, seeds, stems, bark, roots, and latex. Today, many individuals turn to herbal remedies for chronic conditions such as diabetes, cancer, asthma, and liver and kidney damage, often because these options are associated with fewer side effects. Through diverse processes, medicinal plants have produced a variety of phytochemical compounds that provide defence against bacteria, viruses, oxidation, and other environmental stressors. The effectiveness of many traditional herbal remedies has led to their incorporation into modern pharmaceuticals. Numerous plants have shown significant medicinal effects in both pre-clinical and clinical studies. Various medical systems in India, such as Ayurveda, Unani, Homeopathy, Allopathy, and Siddha, utilize plant-based products. Herbal remedies are available in multiple forms, including teas, decoctions, tinctures, elixirs, extracts, essential oils, and inhalations, often made from single plants or combinations, with the plant material serving as the primary ingredient.

1.2 Renal System

The renal system is the main organ system responsible for removing harmful waste products and metabolites from the body. It also plays a key role in regulating blood pH, blood pressure, blood volume, and electrolyte balance.

1.2.1 Nephrons

Each kidney contains over a million nephrons, which are the main functional units of the urinary system. Nephrons contribute to urine formation through processes like tubular secretion, selective reabsorption, and glomerular filtration. A nephron consists of a network of blood capillaries called a glomerulus, located within a cup-shaped structure known as Bowman's capsule. Bowman's capsule then leads into the proximal convoluted tubule (PCT), which continues into the Henle loop, the distal convoluted tubule (DCT), and finally the collecting tubule.

1.2.2 Physiology of urine formation

Kidneys help in the production of urine in 3 phases, i.e., Glomerular filtration, Selective reabsorption, and Tubular secretion.

1.2.2.1 Glomerular filtration

Oxygenated blood flows from the renal artery into an afferent arteriole, which carries it to the glomerulus. The glomerulus and Bowman's capsule, composed of networks of blood capillaries with thin, semipermeable walls, filter blood based on the size of the capillaries and pressure gradients. Large molecules, such as blood cells and proteins, remain in the bloodstream, while smaller molecules like water are filtered out. The net filtration pressure is 35 mmHg. Each day, the two kidneys produce approximately 180 liters of diluted filtrate, of which 1 to 1.5 litres is excreted as urine. Glomerular filtration removes various substances from the blood, including water, electrolytes, creatinine, urea, amino acids, keto acids, uric acid, and drug metabolites, while erythrocytes, leukocytes, thrombocytes, and plasma proteins remain in the glomerulus.

1.2.2.2 Selective tubular reabsorption

Substances are reabsorbed from the renal tubules into the peritubular network through a process called tubular reabsorption. This process involves the absorption of specific materials from the blood, such as glucose, water, electrolytes, and other nutrients. As the glomerular filtrate transitions from the proximal convoluted tubule (PCT) to the distal convoluted tubule (DCT), its volume and composition are altered. This mechanism helps maintain blood pH, glucose levels, fluid balance, and electrolyte balance..

1.2.2.3 Tubular secretion

Urine is produced through the excretion of toxic substances into the distal convoluted tubule (DCT) and the collecting tubule from the peritubular capillaries, bypassing glomerular filtration. Urine concentration increases as tubular secretion occurs, with potassium ions (K^+) and urea being the two main substances released from the blood into the collecting tubules.

1.3 Renal failure

End-stage kidney disease, also referred to as renal failure or kidney failure, is brought on by the loss of kidney function [11]. There are two forms of renal failure: acute kidney failure, which attacks quickly, and chronic kidney failure, which takes time to appear [12].

1.3.1 Chronic Kidney Injury (CKI)

Chronic renal failure (CRF), or chronic kidney insufficiency (CKI), is a medical condition characterized by a gradual, irreversible decline in kidney function. It is commonly caused by nephrotic syndrome, polycystic kidney disease, diabetes, and hypertension. A primary diagnostic marker for CRF is a reduced glomerular filtration rate (GFR) below 60 ml/min. Treatment options include nephrosis, peritoneal dialysis, and haemodialysis.

1.4 Acute Kidney Injury (AKI)

Acute kidney injury (AKI) or acute renal failure (ARF) is a clinical disease in which the kidneys rapidly lose their ability to adequately filter waste products and harmful chemicals from the blood. It is mostly brought on by the kidneys being exposed to nephrotoxic substances. It involves lower concentrations of proteins and urine and increasing concentrations of toxic chemicals such as urea, creatinine, uric acid, BUN, and so on due to lowered GFR [12].

1.4.1 Aetiology

The primary causes of ARF include haemolytic uremic syndrome, low blood pressure, direct kidney injury, muscle damage, changed blood flow to the kidneys, exposure of the kidneys to nephrotoxic medications, and urinary tract blockage [12, 13]. It is commonly recognized that extended exposure to drugs, diagnostic agents such as radiocontrast agents, biological agents like recombinant leucocyte and interferons, and certain substances like heavy metals can result in acute radiation syndrome (ARF) [14]. Table 1 provides information on the medications that induce ARF.

Table 1: List of Nephrotoxic drugs [14, 15]

S.No	Drugs	Example
1	Anti-cancer drugs	Cisplatin, Cyclophosphamide, Doxorubicin, Mitomycin, Mithramycin, Methotrexate
2	Antihypertensives	Valsartan, Trandolapril
3	Anti-microbial agents	Gentamicin, Streptomycin, Amikacin, Tetracycline, Acyclovir, Pentamidine, Sulphadiazine, Trimethoprim, Rifampicin, Amphotericin B
4	Immunosuppressants	Cyclosporin
5	NSAID’s	Paracetamol, Ibuprofen, Indomethacin, Aspirin

1.4.2 Epidemiology

A review of the literature highlights that drug-induced acute renal failure (ARF) is a primary area of study within nephrotoxicity epidemiology. Research indicates that drug-induced ARF impacts approximately 14–26% of adults and about 16% of younger populations. Recent studies show that, while glomerular injury is rare, certain drugs, such as tenofovir, have caused tubular damage in 12–22% of patients receiving treatment. Drug-induced nephrotoxicity is especially prevalent among the elderly due to a range of clinical conditions and extended medication use. Additionally, nephrotoxicity is closely linked to various newer pharmaceutical drugs.

1.4.3 Signs and Symptoms

The clinical symptoms of ARF are as follows :

- Reduced urine output
- Fluid retention,
- which leads to swelling in legs, ankles or feet ¬
- Decreases of breath
- Abnormal heartbeat
- High blood potassium
- Pain in chest and fatigue
- Nausea
- Weakness
- Confusion
- Seizures or coma in severe cases [13]

1.4.4 Pathophysiology of Gentamicin-induced nephrotoxicity

Aminoglycoside antibiotics are commonly used to treat Gram-negative bacterial infections, but they also present significant clinical challenges due to their toxic effects on the kidneys and hearing. Among aminoglycosides, gentamicin is particularly known for its nephrotoxicity. Gentamicin causes acute renal failure (ARF) primarily through mechanisms involving lysosomal phospholipids and tubular necrosis. Evidence suggests that increased calcium levels in the renal cortex and mitochondria are closely linked to cellular necrosis and renal failure associated with gentamicin nephrotoxicity. During gentamicin's metabolism within cells, reactive oxygen species (ROS) and other harmful free radicals are generated, leading to oxidative stress. This includes the production of hydroxyl ions during superoxide synthesis, causing lipid peroxidation and subsequent membrane lipid degradation, which significantly disrupts cell structure and function. Gentamicin exposure also reduces concentrations of protective antioxidants like superoxide dismutase, glutathione, catalase, vitamin E, and ascorbic acid, which typically inhibit oxidative reactions and neutralize ROS. Changes in tubular cell integrity from gentamicin-induced nephrotoxicity may result in either sub-lethal or fatal outcomes. Ultimately, gentamicin induces apoptosis in tubular cells through the intrinsic caspase 8 apoptotic pathway. Tubular necrosis leads to ARF, reducing glomerular filtration rate (GFR) and causing elevated levels of creatinine, uric acid, BUN, and urea, while lowering serum protein, sodium (Na+), and potassium (K+) levels..

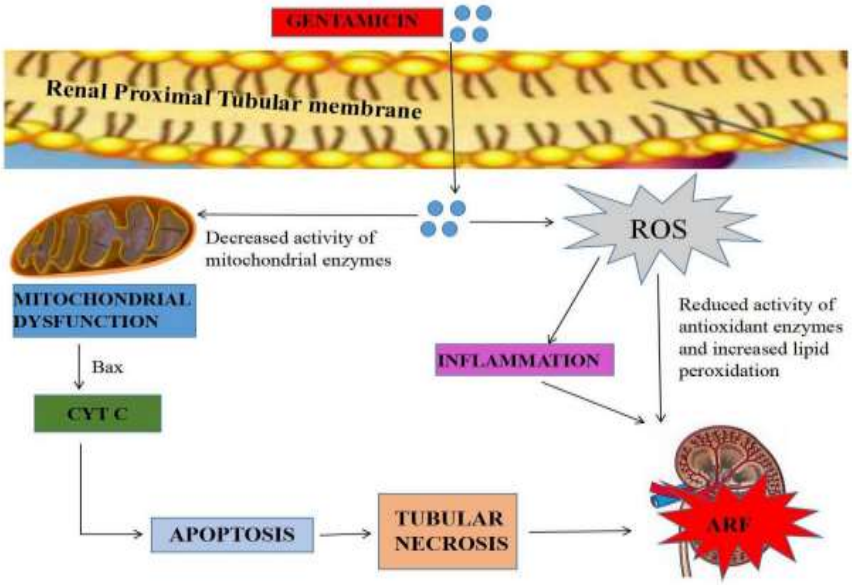

Figure 1: Pathophysiology of Gentamicin-induced nephrotoxicity

1.4.5 Management of ARF

Maintaining volume homeostasis and identifying and treating metabolic imbalances are important goals. Furosemide should be used to treat fluid overload, and alkalis, which aid in dialysis, should be used to treat severe acidosis. Blood transfusions, estrogen or desmopressin administration, and other hematologic problems such as anemia, uremic platelet dysfunction, etc., should be used to treat hyperkalaemia. One key treatment option for AKI is diet restriction. Restricting salt and fluid intake is essential for managing oliguric renal failure [15].

1.4.6 Role of herbals in the treatment of ARF

Due to their high antioxidant content, medicinal herbs are essential for the treatment of AKI. Table 2 lists the several plants that have been shown to have protective Impacts on the kidneys.

2. LITERARURE REVIEW

2.0 PLANT PROFILE

2.1 Biological Source

Botanical Name: *Carica papaya Linn*.

Family: Caricaceae

Parts used: Leaves, flowers, roots, fruits, and Peels.

Description

The papaya is a herbaceous perennial with a lengthy lifespan, that reaches heights of 2 to 10m.it usually has a single ,semi-woody,hollow,erectstem,which terminates with a cluster of large palmately lobed leaves with 25 to 100cm long petioles and latex vessels in all tissues. The papaya life span is 5-10 years.

Occurrence and Distribution

Papaya plant is native to the low land tropical areas of central and south America.it is now grown in topical and subtropical regions around the world. are a plant.

Common names

- ✓ **Hindi**: Papita
- ✓ S**anskrit**: Erand karkati
- ✓ **English**: paw paw

- ✓ **Marathi**:Popay
- ✓ **Gujarati**: papaya
- ✓ **Bengali**: Papeya
- ✓ **Tamil**: Pappali
- ✓ **Telugu**: papay
- ✓ **Malayalam**: omakai
- ✓ **Parsi**: kharboze
- ✓ **Arabi**: babaya or fafay
- ✓ **German**: melonenbaum

2.2 Taxonomic classification

Table 2: Taxonomic classification of *Carica Papaya linn*.

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Sub-class	Dilleniidae
Order	Violales
Family	Caricaceae
Genus	*Carica*
Species	*Carica papaya*

2.7 Morphology

2.7.1 Tree

The pomegranate is a long-lived plant or small tree that reaches heights of 16 to 33 ft. Some examples in France have been known to survive for 200 years

FIGURE 2: Whole plant of CARICA PAPAYA

2.7.2 Leaves

The leaves of carica papaya are star shaped ,with a palmate venation and a parted margin.

FIGURE 3: Leaves of Carica papaya

2.7.3 Fruits

The papayas can also be pear shaped or oval the size of a papaya can range from 15-50 cm long ,10-20 cm thick,and 1-3 kg or more in weight when unripe,papayas have green skin and greenish to white flesh.as they ripen,the skin turns deep yellow or reddish to orange.papayas have small dark gray to black,ovoid,peppery seeds.the flesh of a papaya is juicy and aromatic,and can be yellow,orange,pink or salmon-red colour

FIGURE 4: Fruit of carica papaya

2.7.4 Flowers

The shape of a carica papaya fruit is vary depending on the type of flower that produce it.

- Female flower; The fruit is round or ovoid
- Hermaphrodite flower; The fruit is elongated,cylindrical or pyriform.

FIGURE 5: Flower of Carica papaya

2.8 Nutritional Significance

Carica papaya was proved to have significant nutraceutical properties due to the presence of good amounts of proteins, vitamins, carbohydrates, b vitamins, copper, fibre, potassium, fats, etc [46, 47, 48]. The number and amount of nutrients present in *carica papaya*. were elucidated in Table 3

Table 3: Proximate Composition and Vitamin and Mineral Content of Carica papaya leaves Powder[7–13]

Parameter	**Value**
Proximate Composition (g/100 g)	
Moisture	57.01
Protein	6.50%
Fat	2.01%
Crude fibre	3.10%
Ash	2.18%
Carbohydrates	29.20%
Vitamins (mg/100 g)	
Beta carotene	303.55
Vitamin E	39.78
Vitamin C	68.59
Vitamin B1	199.31
Vitamin B2	295.63
Minerals (mg/100 g)	
Calcium	1086.53
Potassium	30.07
Sodium	30.42
Phosphorus	1971.17
Chromium	31.10

2.9 PHARMACOLOGICAL REVIEW OF ARICA PAPAYA

S.NO	**Extract**	**Plant part used**	**Biological Activity**	**Author and year**
1.	Aqueous and Methanol	Leaves	Antifungal	Priyadarshi et al.,[2018]
2.	Aqueous	leaves	Antibacterial	Sp singh et al.,2020[167]
3.	Methanol	leaves	Anti-inflammatory	NPW yani,N putri[2019]
4.	Ethanol,aqueous	Leaf,flowers	Anti oxidant	MK Dwivedi(2020)
5.	aqueous	Seed,leaf,fruit	Anti microbial	O kadiri(2016)
6.	Aqueous	Leaf extract	Anti microbial	D heena (2019)
7.	Methanol,ethanol,aqueous	Fruit pieces (Peels, pericarp, seeds	Antibacterial	T sharma (2022)
8.	aqueous, and chloroform	fruit	Anti-bacterial	N ismail(2013)
9.	aqueous	leaf	Antiviral	A Qaiser

Table 4: Pharmacological review of *Carica papaya*

2.10 PHYTOCHEMICAL REVIEW OF carica papaya

S.NO	Part	Extract	Constituents	Author and year
1.	leaf	Hexane, ethanol, Methanol, And water	flavonoids, tannins, gallic acids,cynogenic glucosides,saponins,alkaloids	II chinoye., O Cynthia (2000);
2.	leaf	Aqueous	Cardiac glycosides,saponins,tannins,papain,chymopapain,phenols	Tarkang PA.,Agbor GA(2012)
3.	root	Methanol,ethanol, aqueous	Catechin,sapogenin,sparteine,quinine,naringenin,naringin,anthocynaidine,flavone	AM Manzara (2017);037;041
4.	seeds	Aqueous And Methanol	Lipids,crudefiber,sugar,mineral content,seedoil,fattyacid spectrum,tocopherols,carotenoids,benzyl glucosinolates	Peter JQ, Kumar Y, Pandey P;(2014) 9-29.37
5.	Fruit	Methanol, Ethanol, And water	Cysteineprotease enzyme,alkaloids,papin,carpine,pseudocarpine,benzyliso thiocyanate	Nakasone HY, Paul RE 1998.NY,USA

Table 5: Phytochemical review of *CARICA PAPAYA*

3.0 AIMS, OBJECTIVES AND NEED FOR THE STUDY

3.1 Aim :

To evaluate the nephroprotective activity of Chloform carica papaya leaves Extract (CCPLE) in Gentamicin-induced acute renal failure in Wistaralbino rats.

3.2 Objectives :

The following are the research's key targets:

1. Chloroform extraction of the peel of *carica papaya Linn*.
2. Phytochemical screening of *carica papaya Linn*. leaves.
3. An assessment of CCPLE's ability to protect the kidneys from nephrotoxicity caused by Gentamicin.

3.3 Need for the Study:

Drug-induced acute renal failure (ARF) has become increasingly common due to the widespread use of medications for various health conditions. As the primary organ responsible for excretion, the kidneys are highly exposed to these drugs, often leading to renal damage. Despite advancements in medicine, an effective drug treatment for drug-induced kidney damage remains unavailable. Currently, treatment options include surgery and allopathic medicine, both of which carry significant adverse effects, making management challenging. Additionally, these treatments are expensive and require skilled professionals and specialized equipment, prompting researchers to explore herbal remedies with protective effects against renal damage.

A review of the literature reveals that the leaves of the medicinal plant *Carica papaya* Linn. have been traditionally used to treat conditions such as fever, inflammation, tumors, helminthiasis, diarrhea, anorexia nervosa, earache, and others. Studies also show that the leaves contain phytoconstituents like flavonoids, tannins, terpenoids, saponins, and phenolic compounds, which are known for their antioxidant properties. However, the nephroprotective potential of *Carica papaya* Linn. leaves has not yet been scientifically evaluated. This study aims to perform phytochemical screening of the leaves of *Carica papaya* Linn. and assess their nephroprotective activity against drug-induced acute renal failure

3.4 PLAN OF WORK:

4.0 MATERIALS AND METHODS

4.1 Selection of plant

The medicinal plant *carica papaya* Linn. (caricaceae) was selected based on the literature survey.

4.2 Plant collection

The leafs of *carica papaya* were collected in the month of February from papaya gardens.

which is located at sangareddy 70km from JNTUH.

4.3 Authentication

Dr. A. Vijay Bhaskar Reddy, Assistant Professor, Department of Botany, University College of Science, Osmania University, Hyderabad, verified and taxonomically recognized the plant. Osmania University had a voucher specimen of (OUAS-82) in storage.

4.4 Botanical evaluation

Botanical Name	**:**	*carica papaya Linn.*
Family	**:**	caricaceae
Part of the plant used	**:**	leafs

4.5 Determination of Extractive values

Precisely weigh out five grams of finely ground *carica papaya Linn* leafs, then place them into a dry, glass-stoppered conical flask. Macerate for six hours at room temperature, stirring occasionally, with 100 ml of solvent, and let stand for 18 hours. The mixture was quickly filtered, being careful not to lose any of the solvent. A porcelain dish was emptied and 25 ml of the filtrate were evaporated until it was completely dry. After the residue was air-dried for six hours at 105 degrees Celsius, it was measured, and the percentage yield was determined using a formula.

Extractive values= (W1-W2) / Weight of plant powder taken × 100 Here, W1= weight of empty porcelain dish.

W2= weight of porcelain dish with extract.

4.6 EXTRACTION OF PLANT MATERIAL

Successive solvent extraction-soxhlet extraction**:**

Methanol was used to extract compounds from the dried plant material (leaves). Thirty grams of powdered leaves were placed in a thick filter paper thimble and loaded into the main chamber of a Soxhlet extractor. The Soxhlet extractor was placed above a distillation flask containing the methanol

solvent. A condenser was then attached to the Soxhlet extractor to create a closed-loop setup. As the solvent in the distillation flask was heated, its vapors rose through a distillation arm and condensed, dripping into the chamber containing the plant material. The hot solvent gradually filled the chamber with the plant material, dissolving part of the target compounds in the process. When the chamber was nearly full, the siphon tube emptied it back into the distillation flask, allowing the cycle to repeat. This process could continue for several hours or even days until the siphon tube's solvent turned colorless. Finally, the desired compounds were collected by removing the solvent, typically using a rotary evaporator.

Percentage yield is calculated respectively.

$$\text{Percentage yield} = \frac{\text{Weight of crude extract (g)}}{\text{Weight of plant material (g)}} \times 100$$

Figure no.6. Soxhlet extraction process

4.8 SOLVENT RECOVERY BY USING ROTA EVAPORATOR:

A rotatory evaporator is a piece of equipment made expressly to evaporate solvent under vacuum, either in a single stage or through traditional distillation. The evaporator is made up of a heating system and a revolving flask where liquid is poured and readily evaporates to form a papery coating on the heated wall surfaces. The size of the flask, the pressure during distillation, the speed of rotation, and the temperature of the heating system bath all affect how quickly liquid evaporates.

Figure no.7. Solvent recovery by rotatory evaporator

4.9 Qualitative evaluation of phytoconstituents

Many phytoconstituents, including polyphenolic chemicals, carbohydrates, flavonoids, saponins, tannins, and triterpenoids, were presented in the CCPLE through the phytochemical screening tests.

4.9.1 Test for Alkaloids

A) Dragandroff's test

A couple of drops of Dragendroff's reagent (potassium bismuth iodide solution) were applied to one milliliter of filtrates and the development of a noticeable dark brown precipitated was noticed.

B) Mayer's Test

After adding two drops of Mayer's reagent (potassium mercuric iodide solution) to one milliliter of test filtrate in a test tube, a cream-colored deposit was seen.

C) Hager's Test:

Hager's reagent was applied to 1 milliliter of filtrate in a test tube along with two drops, and the result was a yellow precipitate.

D) Wagner's test

To one milliliter of filtrate, two drops of Wagner's reagent (iodine-potassium iodide solution) were applied and inspected for the production of a noticeable reddish brown precipitate.

4.9.2 Test for Carbohydrates

A) Fehling's test

2 ml of CCPLE was added to a mixture consisting of 0.5 ml of Fehling's A and 0.5 ml of Fehling's B solution. After 5 to 10 minutes of heating in boiling water, the combination produced a yellow precipitate that became brick-red, indicating the presence of carbohydrates.

B) Benedict's test

A test tube containing 0.5 ml of CCPLE was filled with 0.5 ml of Benedict's reagent. After five minutes of boiling the mixture, the presence of carbohydrates is indicated by a brick-red precipitate.

C) Molisch's test

A test tube containing 2-3 ml of CCPLE was filled with a few drops of α-naphthol solution. After shaking the test tube, conc. H_2SO_4 was added from the walls. The presence of carbohydrates is shown by a violet ring at the intersection of two liquids.

4.9.3 Test for glycosides

A) Test for cardiac glycosides

Legal's

To CCPLE, 1ml pyridine and 1ml sodium nitroprusside were added. Pink to red colour appears.

B) Test for Saponins glycosides

Foam test

After being forcefully shaken and set aside for three minutes, three milliliters of CCPLE were placed in a test tube. Saponins are present when foam that resembles honeycomb forms.

4.9.4 Test for Flavonoids

A) Shinoda test

A test tube containing 0.5 milliliter of CCPLE was filled with 0.5 milliliter of dilute hydrochloric acid, and then a few pieces of magnesium turnings were added. Colours that are pink or reddish pink indicate the existence of flavonoids.

B) Lead acetate test

One milliliter of CCPLE and one milliliter of lead acetate solution were combined, and the presence of flavonoids is shown by a yellow precipitate.

C) Sodium hydroxide test

To 200mg of CCPLE, 2ml of dil. NaOH was added and formation of yellow colour which gets decolorized on the addition of dil. HCl shows the presence of flavonoids.

4.9.5 Tests for steroids and Terpenoids

A) Salkowski reaction

To 2ml of CCPLE, 2 ml chloroform and 2ml conc. H_2SO_4 was added. Shaken well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

B) Liebermann-Burchard reaction

Chloroform was combined with 2 milliliters of CCPLE. Two drops of concentrated H2SO4 were introduced from the test tube's sidewalls, along with 1-2 milliliters of acetic anhydride. Colours occur in red, blue, and green order.

4.9.6 Test for Tannins and Phenolic compounds

To 2ml of CCPLE, following reagents were added :

- 5% $FeCl_3$ solution ⟶ Deep blue-black colour
- Lead acetate solution ⟶ White precipitate
- Bromine water ⟶ Decolouration of bromine water
- Dil. Iodine solution ⟶ Transient red colour
- Dil. HNO_3 ⟶ Reddish to yellow colour

4.9.7 Test for Proteins and Amino acids

A) Biuret's test

In a test tube, 1 milliliter of heated CCPLE was added, along with 0.5 milliliter of sodium hydroxide (w/v) and 0.1 milliliter of 3% copper sulphate solution. The presence of proteins is indicated by a reddish or violet colour.

B) Million's test

After adding a few drops of Million's reagent to a little amount of plant peel extract, the development of a white precipitate, which denotes the presence of protein, was observed .

C) Ninhydrin test

After heating 3 milliliters of CCPLE, 2-3 drops of a 5% Ninhydrin solution were added. For ten minutes, the mixture was maintained in a bath of boiling water. Amino acids are indicated by their purple or blue colour.

4.10 INVIVO ANTI NEPHROPROTECTIVE STUDIES

4.10.1 Experimental animals

Institutional Animal Ethical Committee (IAEC) has given its approval to the experimental protocol on 9th December 2023 with ethical clearance No: **CPCSEA/1657/IAEC/CMRCP/COL-23/128.** The present investigation employed adults, normal males Albino-Wistar mice weighing between 145 and two hundred grams. Earlier to and throughout the treatment, animals were granted access to drinking water and regular dietary pellets 24 hours a day. The test subjects were made comfortable for a period of seven days in a suitable workplace setting (25oC±1oC temperature, 45-55% RH, unlimited access to food and water) before participating in the study.

4.10.2 Acute Toxicity Studies

According to a review of the research, acute poisoning experiments were conducted on *carica papaya Linn*. leaves using OECD Guidelines 420, Constant Dosage Method, and it was determined to be harmless in animals with doses of as high as 2000mg per kilogram of body [92].

4.10.3 Dose selection

Acute toxicity data make it abundantly evident that the extract was deemed safe and that it was proven to be normal throughout the observation period. Therefore, for the in-vivo experiments, the 1/10th (200 mg/kg body mass) and 1/5th (400 mg/kg body mass) of the (2000 mg/kg body mass) dosage were used.

4.11 Evaluation of Nephroprotective activity

4.11.1 Animal study

The animals were put into five groups of six each.

4.11.2 Treatment

The course of therapy is as follows:

Gr- I-Normal Control: 10 millilitres per kilogram, PO, OD for 21 days. Gr- II: Control of Disease: Gentamicin (80 mg/kg), intraperitoneally, once daily (OD) for eight days. Gr- III - Standard Control: Silymarin (200 mg/KG), po, OD for 21 days, with Gentamicin administered intraperitoneally once daily for the final 8 days. Gr- IV:Test Control (200mg/KG)- CCPLE (200mg/KG), p.o., OD for 21 days. Gentamicin was administered intraperitoneally once a day for the final eight days of Gr-IV:-Test Control (400mg/kg)- CCPLE (400mg/kg), p.o, OD for 21 days.

Gentamicin OD was administered intraperitoneally during the final eight days of Group V:-Test Control (400mg/kg) after CCPLE (400mg/KG) was administered p.o. for 21 days [82].

Figure 8: ANIMAL DOSING

Oral dosing of carica papaya plant extract *Intraperitoneal Administration of Gentamycin to induce nephrotoxicity.*

Body weights were recorded 24 hours after the last dosage was administered, and blood was extracted via the retro-orbital method into Eppendorf tubes. Blood samples were centrifuged at 2000 rpm for ten minutes in order to separate the serum. The biochemical markers such as uric acid, creatinine, urea, (BUN) blood urea nitrogen, globuline, albumin, and entire protein's, among others, were assessed by preparing serum samples. Following blood collection, Animals were killed through having their vertebral columns dislocated, kidneys were removed, and 1 kidney was preserved in ten percent Formalin to prepare histopathological slide. The another kidney was homogenized empolying KCl of ice cold to create homogenation of tissue, which was then used to assess the in vivo antioxidant parameters. [81].

4.11.3 Biochemical evaluation of Serum samples

4.11.3.1 Creatinine

Method: Alkaline picrate method

Principle: When picric acid and creatinine combine in an alkaline media, a red complex is created. The reagent picric acid serves as both a reactant and a deproteinizing agent. An increase in colour intensity denotes a higher creatinine content.

Requirements: Serum sample, Picric acid reagent, Standard, Sodium hydroxide, and Distilled water.

Procedure:

Step-I: Deproteinization of sample

3000µL of the picric acid reagent was added to 500µL of the serum sample. After thoroughly mixed and centrifuged for ten minutes at 3000 rpm, then recovered the supernatant.

Step-II: Colour development

A) Preparation of Test sample :

A mixture of 1000µL of sodium hydroxide and 1750µL of supernatant was prepared.

B) Preparation of Standard :

250µL of standard was prepared by mixing 250µL of sodium hydroxide and 1500µL of the picric acid reagent

C) Preparation of Blank :

1000µL of sodium hydroxide and 1500µL of the picric acid reagent were combined. A total of 1000µL of distilled water was added to this combination, and then mixed well and allowed to stand at room temperature (25ºC) for exactly 20 minutes.Then recorded the absorbance of the Test sample (Abs_{Test}) and Standard ($Abs_{Standard}$) against blank at 520nm within 30 minutes [93].

Calculations :

$$\text{Serum creatinine in mg/dl} = \frac{Abs_{Test} - Abs_{Blank}}{Abs_{Standard} - Abs_{Blank}} \times 2$$

4.11.3.2 Uric acid

Method: Uricase/POD method

Principle : The uricase enzyme converts uric acid into hydrogen peroxide and allantoin. The resulting hydrogen peroxide then couples with 4-aminoantipyrine and aniline derivative to produce a chromogen complex, which is red in colour. Peroxidase is the catalyst for this process. A rise in colour intensity denotes a higher content of uric acid in the sample. The absorbance of the coloured dye is measure at 550 nm.

$$\underset{\text{Uric acid}}{C_5H_4N_4O_3} + \underset{\text{Water}}{2H_2O} \xrightarrow{\text{Uricase}} \underset{\text{Allantoin}}{C_4H_6N_4O_3} + \underset{\text{Water}}{H_2O} + \underset{\text{Carbon-di-oxide}}{CO_2}$$

$$\underset{\text{4-aminoantipyrine}}{C_{11}H_{13}N_3O} + \underset{\text{Water}}{H_2O} + \underset{\text{derivative}}{\text{Aniline}} \xrightarrow{\text{Peroxidase}} \underset{\text{(Red color)}}{\text{Qiunoneimine dye}} + \underset{\text{Water}}{H_2O}$$

Requirements: Serum sample, Uric acid Mono reagent, Uric acid Standard, and Distilled water.

Procedure :

B) Preparation of Test sample :

A mixture of 20µL of serum sample and 1000µL of uric acid mono reagent (Reagent 1) was prepared.

Preparation of Standard :

A mixture of 20µL of uric acid standard (Reagent 2)and 1000µL of uric acid mono reagent (Reagent 1) was prepared.

D) Preparation of Blank :

After thoroughly mixing 20µL of distilled water with 1000µL of uric acid mono reagent (Reagent 1), the mixture was let to remain at room temperature (25oC) for ten minutes. within thirty minutes, we measured the absorbance of the Standard sample (AbsStandard) and Test sample (AbsTest) at 550 nm against blank [94].

Calculations :

$$\text{Uric acid in mg/dl} = \frac{Abs_{Test}}{Abs_{Standard}} \times 6$$

4.11.3.3 Urea

Method: Modified Berthelot method

Principle: The urease enzyme breaks down urea into ammonia and carbon dioxide. The resulting ammonia then reacts with hypochlorite and phenolic chromogen to generate a green-coloured complex. An increase in colour intensity denotes a higher urea content in the sample.

$$\underset{\text{Urea}}{CH_4N_2O} + \underset{\text{Water}}{H_2O} \xrightarrow{\text{Urease}} \underset{\text{Ammonia}}{2NH_3} + \underset{\text{Carbon-di-oxide}}{CO_2}$$

$$\text{Ammonia} + \text{Phenolic chromogen} + \text{Hypochlorite} \longrightarrow \text{Green colored complex}$$

Requirements: Serum sample, Buffer reagent (L_1), Enzyme reagent (L_2), Chromogen reagent (L_3), Urea standard (S), and Distilled water.

Procedure:

A) Preparation of Test sample :

100µL of buffer reagent (L_1) was added to 100µL of enzyme reagent (L_2). To this mixture, added 10µL of serum/urine sample.

B) Preparation of Standard :

100µL of buffer reagent (L_1) was added to 100µL of enzyme reagent (L_2). To this mixture, added 10µL of urea standard (S).

C) Preparation of Blank :

100µL of enzyme reagent (L2) and 100µL of buffer reagent (L1) were combined. After adding 10µL of distilled water to the mixture, stirring thoroughly, and letting it stand at room temperature (25oC) for ten min, 200µL of Chromogen reagent (L3) was added to each preparation, stirred thoroughly, and allowed to stand at room temperature (25oC) for ten minutes. Within 60 minutes of recording the absorbance of the Standard (AbsStandard) and Test sample (AbsTest) against blank at 570 nm [95, 96].

Calculations :

$$\text{Urea in mg/dl} = \frac{\text{Abs}_{\text{Test}}}{\text{Abs}_{\text{Standard}}} \times 40$$

$$\text{Blood Urea Nitrogen(BUN) in mg/dl} = \text{Urea in mg/dl} \times 0.467$$

4.11.3.4 Total protein

Method: Biuret method

Principle: In an alkaline media, proteins bond to cupric ions in the biuret reagent to generate a complex with a blue-violet color. A rise in color intensity denotes a higher protein content in the sample.

$$\textbf{Proteins} + \textbf{Cu}^{+2} \xrightarrow{\text{Alkaline medium}} \textbf{Blue-violet colored complex}$$

Requirements: Serum sample, Biuret reagent (L_1), Protein standard (S), and Distilled water.

Procedure :

A) Preparation of Test sample :

A mixture of 20µL of serum sample and 1000µL of biuret reagent (L_1)was prepared.

B) Preparation of Standard :

A mixture of 20µL of protein standard(S) and 1000µL of biuret reagent (L_1) was prepared.

C) Preparation of Blank :

After thoroughly mixing 1000µL of biuret reagent (L1) with 20µL of distilled water, the mixture was let to remain at room temperature (25oC) for half an hour. and after that, within 60 minutes, measured the absorbance of the Standard (AbsStandard) and Test sample (AbsTest) at 550 nm in comparison to the blank [96].

Calculations :

$$\text{Total proteins in g/dl} = \frac{\text{Abs}_{\text{Test}}}{\text{Abs}_{\text{Standard}}} \times 8$$

4.11.3.5 Albumin

Method: BCG method

Principle: When albumin binds to bromocresol green dye, a green complex is created. An increase in color intensity denotes a higher protein content in the sample.

$$\textbf{Albumin + Bromocresol green} \xrightarrow{\text{Buffered medium}} \textbf{Blue-violet colored complex}$$

Requirements: Serum sample, BCG reagent (L_1), Albumin standard (S), and Distilled water.

Procedure :

A) Preparation of Test sample :

A mixture of 10µL of serum sample and 1000µL of BCG reagent (L_1) was prepared.

B) Preparation of Standard :

A mixture of 10µL of standard sample and 1000µL of BCG reagent (L_1) was prepared.

C) Preparation of Blank :

After thoroughly mixing 10µL of distilled water with 1000µL of BCG reagent (L1), the mixture was let to remain at room temperature (25oC) for five minutes. Using a UV-Visible spectrophotometer, the absorbance of the Test sample (AbsTest) and Standard (AbsStandard) was measured at 630 nm in relation to a blank [96].

Calculations :

$$\text{Albumin in g/dl} = \frac{\text{Abs}_{\text{Test}}}{\text{Abs}_{\text{Standard}}} \times 8$$

$$\text{Globulin in g/dl} = \text{Total proteins (in g/dl)} - \text{Albumin (in g/dl)}$$

4.11.4 Histopathological Studies

The detached kidneys, which had been kept in ten per cent formalin, were imbedded in wax made of paraffin and longitudinal cut using a microtome cutters. They had been stained with haematoxylin and eosin (H&E) and examined via a trinocular microscope [81].

4.11.5 Statistical analysis

All findings were evaluated utilizing one way analysis of variance (ANOVA) & Dunnett's multiple comparison test (DMCT) [100].

5.RESULTS AND DISCUSSION

5.1 Results

5.1.1 EXTRACTIVE VALUES:

Extractive values were determined by using standard procedures and values were tabulated in table.

S.NO	**SOLVENTS**	**EXTRACTIVE VALUE**
1.	Water	28% (w/w)
2.	Methanol	15.2% (w/w)
3	Acetone	7.2% (w/w)
4	Hexane	6.4%(w/w)
5	Chloroform	32%(w/w)

Table:6 Solvent extractive values of leaves of *carica papaya* **with different solvents (%w/w).**

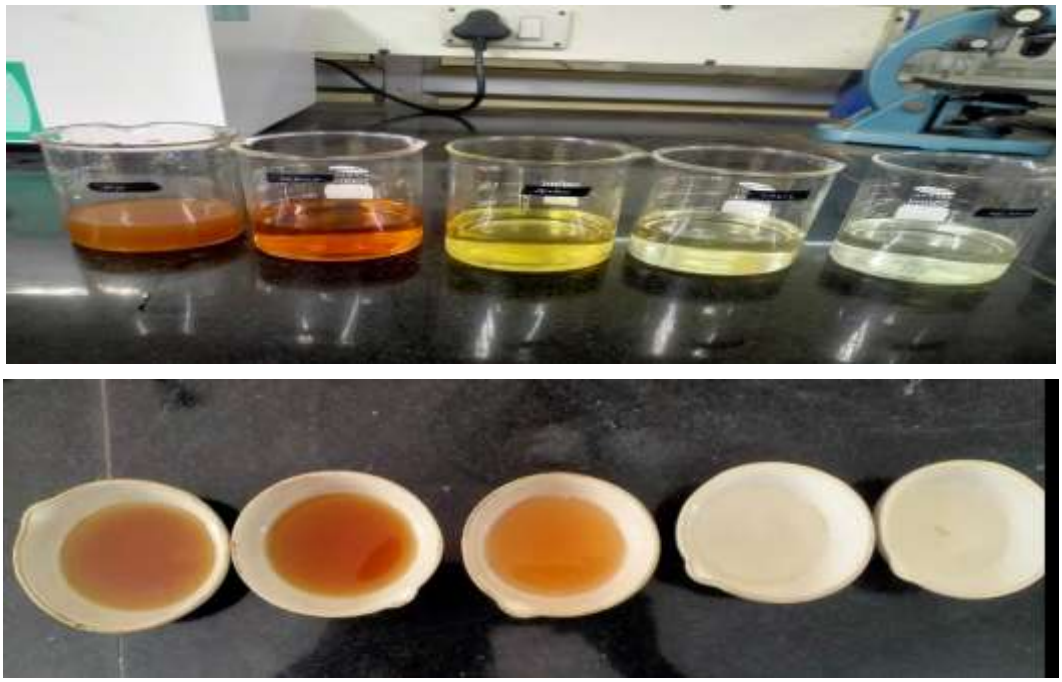

Figure 9: Air-dried methanol, water, acetone, hexane, and chloroform extracting properties.

Water (28% W/w), Methanol (15.2% W/w), Acetone (7.2% of the W/w), Hexane (6.4% W/w), & chloroform (10.4 %W/w) were determined to have the greatest extractive values according to the table data above. Methanol was chosen for additional extraction since water has the greatest extractive yield of all the samples, which is 14% W/w.

5.1.2 Preliminary Phytochemical screening

Results of phytochemical screening were elucidated in Table-11.

Table 7: Results of Phytochemical screening

S. No	Phytochemical	Observation
1.	Alkaloids	+
2.	Flavonoids	+
3.	Tannins	+
4.	Saponins	+

5.	Steroids	+
6.	Amino acids	+
7.	Carbohydrates	+
8.	Glycosides	+

Where, (+) means Positive and (-) means Negative.

The initial phytochemical analysis revealed that CCPLE included a variety of phytoconstituents, including proteins, carbohydrates, tannins, saponins, alkaloids, phenolic compounds, flavonoids, and glycosides. Amino acids were not present in CCPLE.

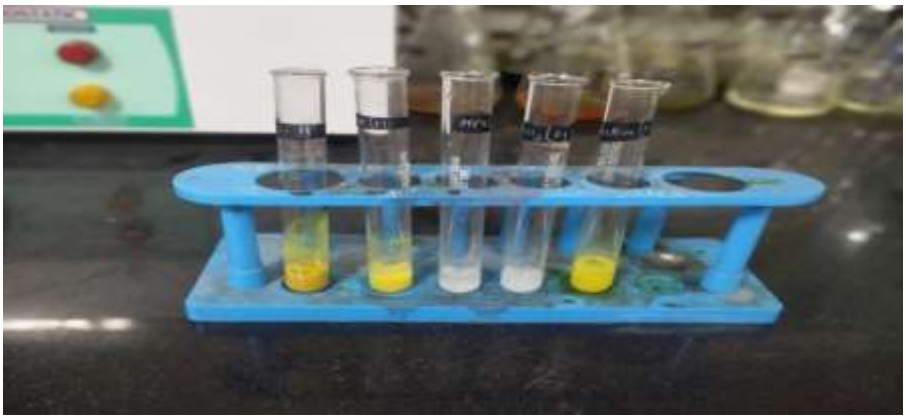

Figure 10 : Preliminary Phytochemical screening

5.1.3 Body weights

Table 8: Change in Body weights on 1st Day and 21th Day

S.No	**Treatment Groups**	**Body weights on Day 1 (in grams)**	**Body weights on Day 21 (in grams)**
1.	Normal control	156.15±6.060	158.82±6.091
2.	Disease control	162.40±8.067	165.30±6.071
3.	Standard control	175.02±6.041	237.20±8.128
4.	CCPLE 100mg/Kg	175.50±6.065	235.22±8.220
5.	CCPLE 200mg/Kg	165.02±6.076	212.11±8.076

Values are represented as Mean ± SD.

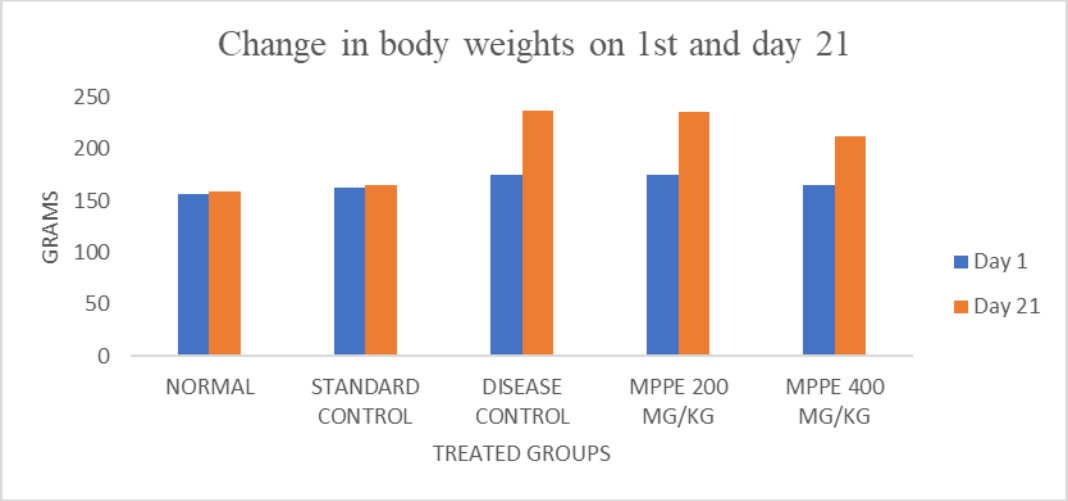

Figure 11: Change in Body weights on 1st and 29th Day

On Day 21, it was discovered that the significant readings of body weight of CCPLE 200 mg per kilogram, CCPLE 400 mg per kilogram, illness, normal, and standards were 156.15±6.060,162.40±8.06,175.02±6.041,175.50±6.065 and 165.02±6.076respectively on Day 1 and 158.82±6.091, 165.30±6.071, 237.20±8.128, 235.22±8.220 and 212.11±8.076 respectively When compared to disease control, animals administered CCPLE 200 mg per kilogram and 400 mg per kilogram had significantly greater body weight.

5.14 Serum parameters

Table 9: Impact of CCPLE on Serum parameters

S. No	Treatment Groups	Creatinine (mg/dl)	Uric acid (mg/dl)	Urea (mg/dl)	BUN (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
1.	Normal Control	0.643± 0.0416	3.185± 0.295	35.460 ±4.013	18.040 ±1.890	16.200 ±0.564	9.128± 0.205	8.267± 0.440
2.	Disease Control	1.485± 0.0421	5.792± 0.325	110.12 ±4.983	53.950 ±2.341	4.927± 0.448	5.583± 0.314	1.343± 0.209
3.	Standard control	0.712± 0.0448***	3.335± 0.216***	47.640 ±4.020***	22.780 ±1.872***	9.843± 0.484***	5.998± 0.350***	3.855± 0.418***
4.	CCPLE 200mg/ kg	0.754± 0.0389***	3.805± 0.268***	51.010 ±5.868***	25.352 ±2.745***	9.485± 0.581***	5.868± 0.484***	3.647± 0.466***
5.	CCPLE 400mg/ kg	0.728± 0.0268***	3.656± 0.195***	53.721 ±3.159**	22.690 ±1.471**	8.967± 0.485***	5.585± 0.255**	3.682± 0.341***

Values are represented as Mean ± SEM. Statistical analysis was done by one way ANOVA followed by post hoc Dunnett's multiple comparison tests. ***p<0.0001,**p<0.001, and *p<0.05 vs Disease control.

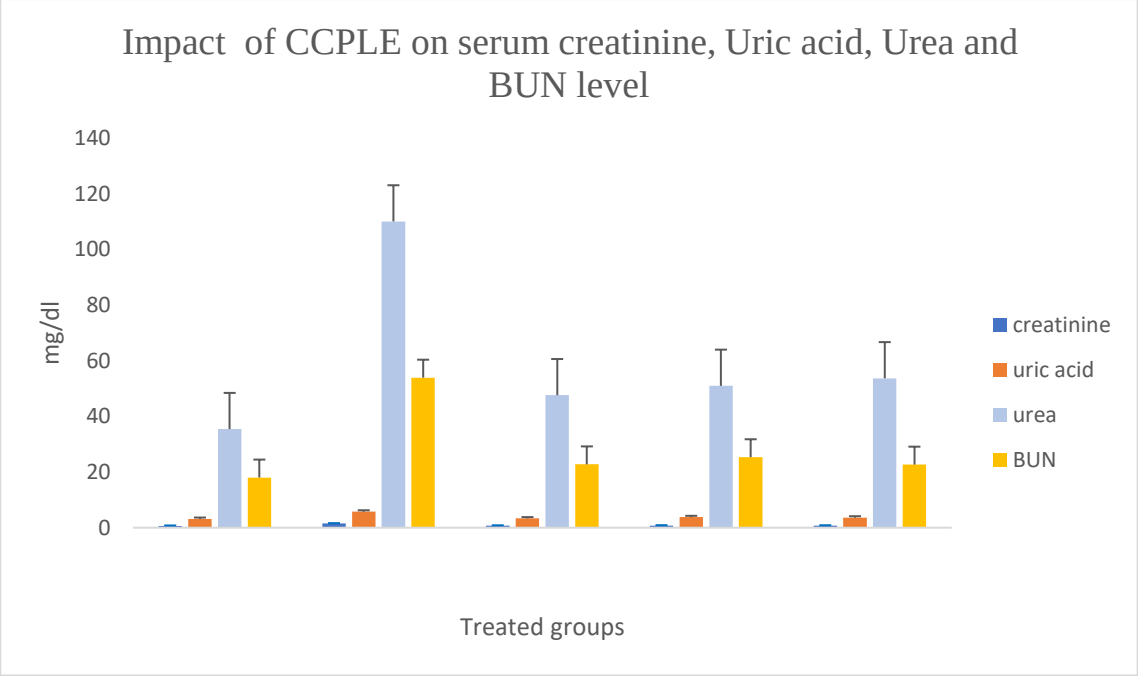

Figure 12: Impact of CCPLE on serum Creatinine, Uric acid, Urea and BUN level

5.1.4.1 Impact of CCPLE on Creatinine concentration

On Day 21, it was discovered that the significant readings of creatinine concentration of CCPLE 200 mg per kilogram, CCPLE 400 mg per kilogram, illness, normal, and standards were 0.654±0.0416, 1.485±0.0421, 0.712±0.0448, 0.754±0.0389 and 0.728±0.0268 respectively. When compared to disease control, animals administered CCPLE 200 mg per kilogram and 400 mg per kilogram had significantly lower creatinine concentration.

5.1.4.2 Impact of CCPLE on Uric acid concentration

On Day 21, it was discovered that the significant readings of serum uric acid concentration of CCPLE 200 mg per kilogram, CCPLE 400 mg per kilogram, illness, normal, and standards were 3.185±0.295, 5.792±0.325, 3.335±0.216, 3.805±0.268, and 3.656±0.195, respectively. When compared to disease control, animals administered CCPLE 200 mg per kilogram and 400 mg per kilogram had significantly lower serum uric acid concentration.

5.1.4.3 Impact of CPPLE on Urea concentration

On Day 21, it was discovered that the significant readings of serum urea concentration of CCPLE 200 mg per kilogram, CCPLE 400 mg per kilogram, illness, normal, and standards were 35.460±4.013, 110.12±4.983, 47.640±4.020, 51.010±5.868 and 53.721±3.159 respectively. When compared to disease control, animals administered CCPLE 200 mg per kilogram and 400 mg per kilogram had significantly lower urea concentration.

5.1.4.4 Impact of CCPLE on BUN concentration

On Day 21, it was discovered that the significant readings of BUN concentration of CCPLE 200 mg per kilogram, CCPLE 400 mg per kilogram, illness, normal, and standards were 18.040±1.890, 53.950±2.341, 22.780±1.872, 25.352±2.745 and 22.690±1.471 respectively. When compared to disease control, animals administered CCPLE 200 mg per kilogram and 400 mg per kilogram had significantly lower BUN concentration.

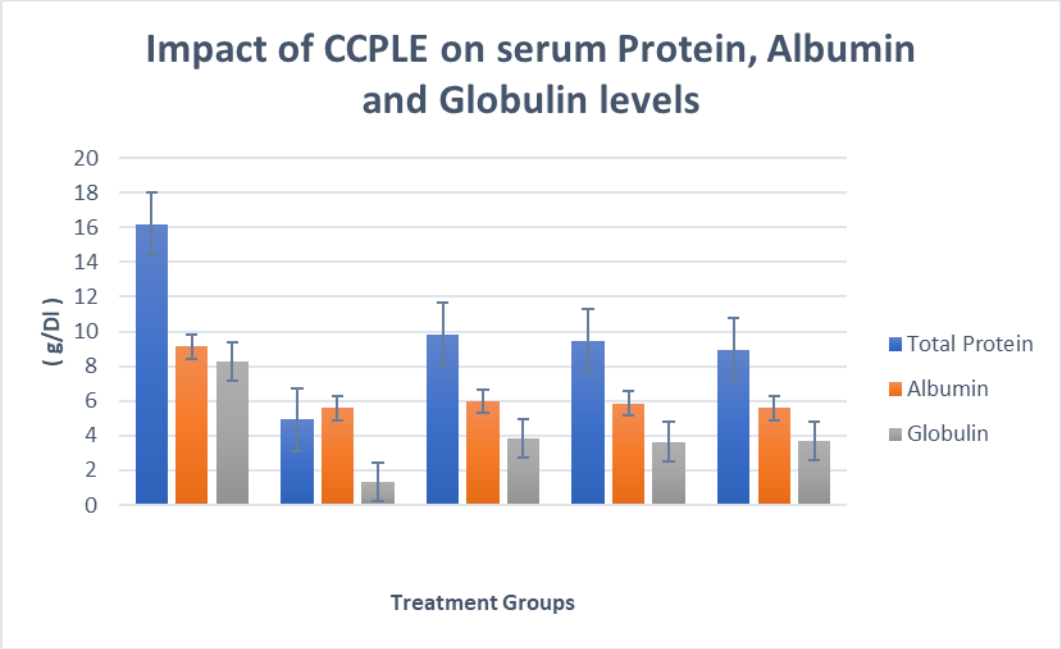

Figure 13 : Impact of CPPLE on serum Protein, Albumin and Globulin concentration

5.1.4.5 Impact of CCPLE on Protein concentration

On Day 21, it was discovered that the significant readings of serum Protein concentration of CCPLE 200 mg per kilogram, CCPL E 400 mg per kilogram, illness, normal, and standards were 16.200±0.564, 4.927±0.448, 9.843±0.484, 9.485±0.581 and 8.967±0.485 respectively. When compared to disease control, animals administered CCPLE 200 mg per kilogram and 400 mg per kilogram had significantly higher protein concentration.

5.1.4.6 Impact of CCPLE on the Albumin Concentrations

On Day 21, it was discovered that the significant readings of serum Albumin concentration of CCPLE 200 mg per kilogram, CCPLE 400 mg per kilogram, illness, normal, and standards were 9.128±0.205, 5.583±0.314, 5.998±0.350, 5.868±0.484 and 5.585±0.255 respectively. When compared to disease control, animals administered CCPLE 200 mg per kilogram and 400 mg per kilogram had significantly higher albumin concentration.

5.1.4.7 Impact of CCPLE on Globulin concentration

On Day 21, it was discovered that the significant readings of serum globulin concentration of CCPLE 200 mg per kilogram, CCPLE 400 mg per kilogram, illness, normal, and standards were 8.267±0.440, 1.343±0.209, 3.855±0.418, 3.647±0.466 and 3.682±0.341 respectively. When compared to disease control, animals administered CCPLE 200 mg per kilogram and 400 mg per kilogram had significantly higher globulin concentration.

5.1.5 Histopathological studies

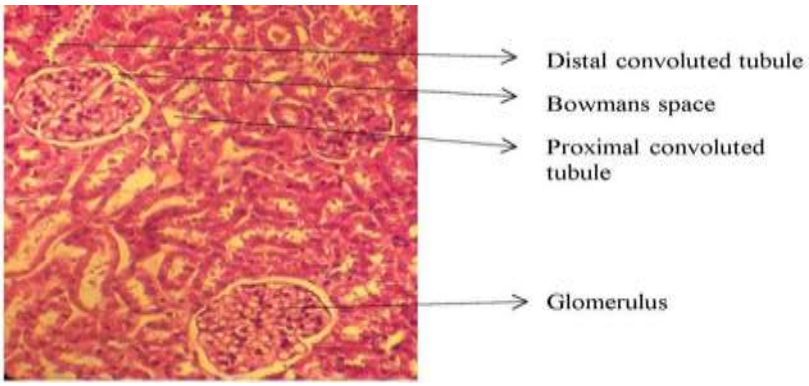

Figure 13 (a) (Saline 10ml/kg)

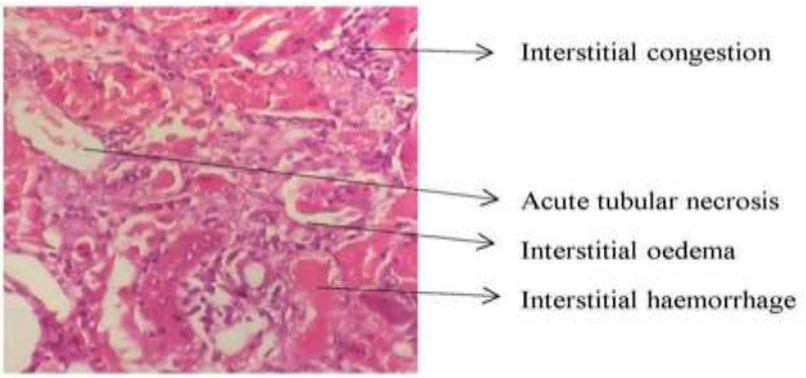

Figure 14 (b) (Gentamicin 80mg/kg)

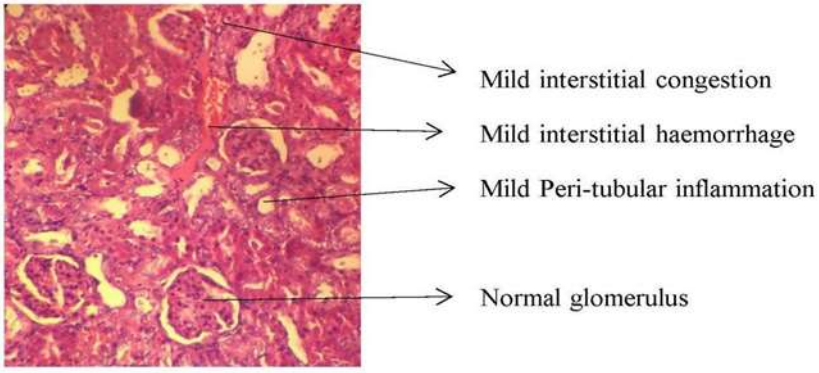

Figure 15 (c) (Sylimarine)

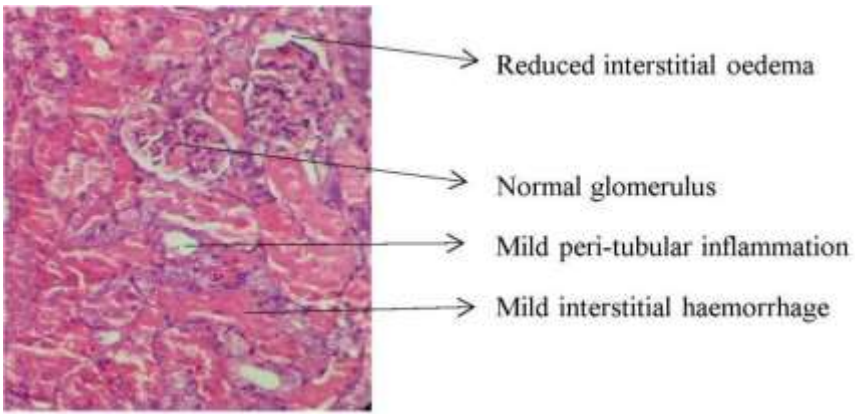

Figure 16 (d) (CCPLE 200mg/kg)

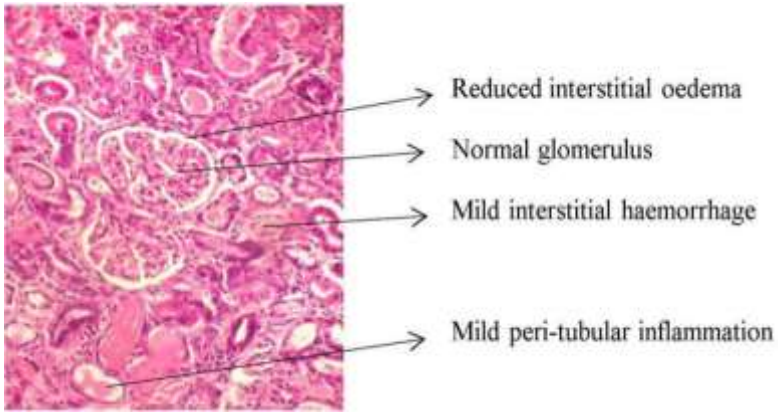

Figure 17 (e) (CCPLE 400mg/kg)

5.2 DISCUSSION

The current study reports botanical compounds and pharmacology evaluations of the kidney-protective activity of an extract made with methanol of pomegranate peel at dosages of two hundred and four hundred mg per kilogram total body weight. CCPLE contains flavonoids, alkaloid compounds, the saponins, sugars, proteins, the tannins, and phenolic substances, according to initial phytochemical studies.

Gentamicin renal toxicity is one of the main causes of sudden kidney failure. Numerous investigations have confirmed how oxidative stress contributes to kidney injury. Prior research indicates a high possibility that reactive oxygen species (ROS) mediate gentamicin's effects on the kidneys. Furthermore, ROS contribute to gentamicin-induced proximal tubular necrosis and acute renal failure.

Gr- I-Normal Control: 10 millilitres per kilogram, PO, OD for 21 days. Gr- II: Control of Disease: Gentamicin (80 mg/kg), intraperitoneally, once daily (OD) for eight days. Gr- III - Standard Control: Silymarin (200 mg/KG), po, OD for 21 days, with Gentamicin administered intraperitoneally once daily for the final 8 days. Gr- IV: Test Control (200mg/KG)- CCPLE (200mg/KG), p.o., OD for 21 days. Gentamicin was administered intraperitoneally once a day for the final eight days of Gr-IV:-Test Control (400mg/kg)- CCPLE (400mg/kg), p.o, OD for 21 days. Gentamicin OD was administered intraperitoneally during the final eight days of Group V:-Test Control (400mg/kg) after CCPLE (400mg/KG) was administered p.o. for 21 days

One common aminoglycoside antibiotic that is known to have considerable nephrotoxic potential in both people and laboratory animals is gentamicin (GM). Increased plasma concentration of uric acid, creatinine, urea, and blood urea nitrogen are indicative of GM-induced nephrotoxicity. Serum parameters such as creatinine metabolism, uric acid, urea, blood urea nitrogen, protein, globulin and albumin were examined as part of the nephroprotective research to evaluate the nephroprotective efficacy of CCPLE. The parameters for serum and urine are dependent on two things. The GFR and the rate of tubular re-absorption are the two. One possible explanation for the observed impact is an higher in renal blood flow, which in turn leads to an greater in GFR.

The current investigation shows that CCPLE considerably raised GFR in comparison to animals in the disease control group. CCPLE raised blood protein, albumin, and globulin while lowering serum creatinine, uric acid, urea, and BUN. These findings suggest that CCPLE is improving renal function.

The observed histological data revealed a strong correlation with the biochemical results, Both the standard and CCPLE therapy groups showed substantial improvements, when compared with the disease group that received no treatment.

As a result, it has been proven that the plant *carica papaya Linn.* has nephroprotective effect towards Gentamicin- induced sudden renal damage.

6.SUMMARY AND CONCLUSION

carica papaya leaves were gathered, authenticated, shade dried, and extracted, with extractive values determined using chloroform, methanol, hexane, acetone and water. The extracts' respective dry crushed leaves materials were used to give the following % yields: methanol (11.8% w/w), water (14% w/w), acetone (3.2% w/w), hexane (0.6% w/w), and chloroform (0.2% w/w). The extracts were then submitted to initial phytochemical testing utilizing traditional methods of analysis. In the case of phytochemical testing, the build-up of phytochemicals was determined to be higher in the methanol solvent, while the yield as a percentage of the water extract was greater when compared to the remaining solvents, namely water and methanol. Hence methanol solvent was utilized for the extraction technique.

The crushed peeled materials were extracted using the soxhlation procedure for four hours, with methanol as the solvent. *carica papaya* had a percentage yield that was 24.66% w/w. Following the finish of extraction. The desiccated CCPLE was properly preserved for phytochemical examination & kidney-protective investigations.

Alkaloids, sugars, proteins, phenolic chemicals, tannins, terpenoids, saponins, and flavonoids were detected by phytochemical examination of CCPLE. Based on the literary works, CCPLE was proven to be harmless at the level utilized, with no reported deaths below the 2000 milligrams per kilogram of dosage. CCPLE also demonstrated considerable protective efficacy against ARF produced by Gentamicin. CCPLE's reno-protective action might be attributed to flavonoids, terpenoids, saponins, tannins, and phenolic substances, either alone or in combination. However, there is a considerable likelihood that flavonoids or tannins demonstrated significant nephroprotective efficacy since they have a positive effect on the urinary system, according to the research.

Hence, we may infer that the leaves of pomegranates have nephroprotective properties.