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Research Article

Evaluation Of Diuretic Activity of Ethanolic Leaf Extract of Morus Alba in Albino Rat

Jyothi B. N.*, Kiran K. J., Sanitha M., Shabin P., Liya Saji, Akshaya P., Prasobh G. R.

Sree Krishna college of Pharmacy and Research Centre Parassala, Thiruvananthapuram.

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ABSTRACT

This study evaluated the diuretic potential of the ethanolic leaf extract of Morus alba (white mulberry), a plant traditionally used for urinary and kidney disorders. The leaves were shade-dried, powdered, and extracted with 70% ethanol. Phytochemical screening confirmed the presence of flavonoids, phenols, and tannins, with a total flavonoid content of 42.82 mg/g. Docking studies showed that major compounds like isoquercitrin, moracetin, morin, and rutin had strong binding with diuretic target proteins (carbonic anhydrase II and vasopressin receptor 2). In vitro assays indicated carbonic anhydrase inhibition, and in vivo studies in Wistar rats (by the Lipschitz method) demonstrated a dose-dependent increase in urine volume, pH, and electrolyte excretion, similar to the standard drug furosemide. These results suggest that Morus alba possesses significant diuretic activity and may serve as a safe, natural alternative to synthetic diuretics.

INTRODUCTION

Diuretics are substances that promote the excretion of excess water and salts through increased urine formation, helping to regulate fluid balance in the body. They are commonly prescribed for conditions such as hypertension, congestive heart failure, liver cirrhosis, and kidney disorders.^[1] These agents act on different parts of the nephron to reduce sodium and water reabsorption, thereby enhancing urine output. Major categories include

loop diuretics, thiazides, potassium-sparing diuretics, and osmotic agents.^[2] Although highly effective, conventional synthetic diuretics may produce adverse effects like disturbances in electrolyte levels, dehydration, or kidney injury, which has driven growing interest in plant-derived alternatives that are safer and more affordable.^[3] While diuretics provide significant therapeutic benefits, they can lead to side effects depending on the type used and the dosage administered. Common complications include electrolyte imbalances such as hypokalaemia, hyperkalaemia,

*Corresponding Author: Jyothi B. N.

Address: Sree Krishna college of Pharmacy and Research Centre Parassala, Thiruvananthapuram.

Email : Jyothinarayanan2000@gmail.com

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hyponatraemia, and either metabolic alkalosis or metabolic acidosis. Excessive or inappropriate use of diuretics can also cause dehydration and decreased kidney function, making it essential to monitor electrolyte levels and renal performance throughout treatment.

In patients with chronic health problems such as heart failure or chronic kidney disease, diuretics are fundamental in managing excess fluid volume. For example, loop diuretics are often given to relieve symptoms of fluid overload in heart failure, thereby improving quality of life and reducing hospital admissions.^[4] When resistance to diuretic therapy occurs, combining loop and thiazide diuretics can create a synergistic effect to overcome diminished response. Diuretics also play a role in conditions other than fluid overload. Thiazide diuretics, for instance, are useful in idiopathic hypercalciuria and in preventing kidney stone formation because they reduce urinary calcium excretion.^[5] Spironolactone is increasingly prescribed for disorders such as polycystic ovary syndrome and resistant hypertension because of its anti-androgenic actions. The specific diuretic chosen depends on the clinical situation, kidney function, and individual patient characteristics. For people with hypertension who also have osteoporosis, thiazide diuretics may be preferred for their calcium-sparing benefits, whereas patients with reduced renal function or acute heart failure may gain more from loop diuretics.^[6]

Diuretics are among the most widely prescribed drugs in medical practice, providing vital control of cardiovascular, renal, and endocrine conditions.^[7] By promoting sodium loss (natriuresis) and thereby water loss (diuresis), they reduce plasma volume and blood pressure in those with hypertension. Their mechanisms differ depending on their site of action within the kidney. Loop

diuretics act in the thick ascending limb of the loop of Henle by inhibiting a protein that reabsorbs roughly a quarter of filtered sodium, making them potent agents for rapid fluid removal.^[8] Thiazides inhibit sodium reabsorption in the distal convoluted tubule, which normally reabsorbs about five percent of sodium, making them less powerful but effective for long-term blood pressure management.^[9] Pharmacokinetics also influence their use: for example, loop diuretics such as furosemide have short half-lives and may require multiple daily doses, whereas thiazide-like drugs like chlorthalidone have longer half-lives and provide steadier blood pressure control over 24 hours.^[10] In clinical practice, diuretics are often combined with other antihypertensive drugs such as ACE inhibitors or calcium channel blockers to enhance blood pressure reduction and reduce side effects like hypokalaemia. Combining thiazides with potassium-sparing agents helps maintain potassium balance and lowers the risk of arrhythmias in susceptible patients.^[11]

Certain groups respond better to specific diuretics. Elderly individuals and African American patients with hypertension often respond more effectively to thiazide diuretics, while patients with liver cirrhosis and ascites usually receive spironolactone as a first-line treatment due to its ability to block aldosterone and counter sodium retention.^[12] Despite their benefits, diuretics require careful use because of potential adverse effects. Electrolyte disturbances such as hypokalaemia, hyperuricemia leading to gout, and hyponatraemia are common with thiazides.^[13] Loop diuretics can cause hearing damage, particularly when given intravenously at high doses or together with other ototoxic medicines like aminoglycosides.^[14] Therefore, individuals on diuretic therapy need regular monitoring of kidney function and serum electrolytes. Resistance to diuretics, particularly loop agents, can develop in



patients with chronic kidney disease or heart failure.^[15] This “diuretic resistance” often requires sequential nephron blockade, where a loop diuretic is combined with a thiazide to improve response. Managing this condition also involves addressing underlying factors such as low kidney blood flow, use of NSAIDs, or excessive dietary sodium intake.^[16]

Beyond cardiovascular and renal indications, diuretics have specialized uses. Acetazolamide is employed not only for glaucoma but also to induce metabolic acidosis in treating metabolic alkalosis and to prevent acute mountain sickness.^[17] Mannitol, an osmotic diuretic, is used in neurocritical care to decrease intracranial pressure after traumatic brain injury or stroke. Current research is focusing on discovering new diuretic agents and developing personalized treatments based on pharmacogenomics. Genetic differences in sodium transporter proteins can influence how patients respond to diuretics, paving the way for tailored therapies in hypertension and heart failure. Sodium-glucose co-transporter 2 (SGLT2) inhibitors, first developed for diabetes, show mild diuretic action and cardiovascular benefits, representing a newer class of diuretic-like drugs.^[18] Overall, diuretics remain central to modern therapeutics, and careful selection and dosing tailored to the patient are key to achieving treatment goals while minimizing side effects. Ongoing investigations and emerging drug classes continue to expand their role in clinical medicine.^[19]

Traditional healing systems have relied on herbal remedies for centuries, and in recent years scientific attention has increasingly turned to medicinal plants as potential sources of novel therapeutic agents.^[20] Among these, diuretic herbs are of particular interest because they may provide natural alternatives with fewer side effects. Many

plants are rich in bioactive compounds such as flavonoids, alkaloids, tannins, and saponins that can produce diuretic effects. Evaluating these plants in experimental models helps validate their traditional use and may lead to the discovery of new medicines.^[21]

One promising plant is *Morus alba*, commonly known as white mulberry, a member of the Moraceae family that originated in China but is now widely grown in India and many other regions. Traditionally, its leaves, fruits, bark, and roots have been used to treat numerous health problems.^[22] The leaves, in particular, contain abundant flavonoids, polyphenols, and alkaloids and show a range of pharmacological activities including antidiabetic, antioxidant, antimicrobial, liver-protective, and blood-pressure-lowering effects. Several investigations have indicated that ethanolic extracts of *Morus alba* leaves contain compounds that can affect blood pressure and kidney function, making the plant a strong candidate for exploring diuretic potential. Because the plant is readily available, affordable, and environmentally sustainable, it offers an attractive option for natural drug discovery, especially for populations where access to synthetic drugs is limited.^[23]

Despite the long-standing use of *Morus alba* leaves in traditional medicine for kidney and urinary disorders, scientific proof of its diuretic activity is still limited. It is therefore important to examine its effects using established experimental models.^[24] The albino rat is frequently used in pharmacological studies because of its sensitivity and physiological similarity to humans. Ethanolic extracts of *Morus alba* leaves are especially valuable because they capture a wide spectrum of polar and non-polar compounds that may enhance biological activity. Investigating its influence on urine output, sodium, potassium, and chloride

excretion, as well as kidney function, can provide significant insights into its therapeutic potential. The kidneys maintain internal balance by regulating body fluids, electrolytes, acid–base levels, and waste elimination. Nephrons, the functional units of the kidney, filter blood and selectively reabsorb or secrete substances to form urine. ^[25] Diuretics disrupt these processes by interfering with sodium and chloride reabsorption at various points in the nephron, which changes osmotic gradients and limits water reabsorption, thereby increasing urine volume.^[26]

Many plant extracts imitate the action of conventional diuretics by modifying renal blood flow, altering the glomerular filtration rate, or interacting with specific ion transport mechanisms. Bioactive phytochemicals like flavonoids, glycosides, tannins, and alkaloids are thought to act either directly on the nephron or indirectly by affecting hormones such as aldosterone or antidiuretic hormone. ^[27] Plants like *Morus alba* may also exert mild anti-inflammatory, antioxidant, and blood-pressure-lowering effects that indirectly improve kidney function and enhance diuresis. *Morus alba* has a long history of use in traditional medical systems such as Ayurveda, Siddha, and Chinese medicine and is typically recommended for ailments including

- oedema (fluid retention)
- urinary tract infections
- hypertension, diabetes
- kidney dysfunction
- **Anatomy of kidney**

The kidneys are a pair of bean-shaped organs situated in the retroperitoneal space on both sides of the vertebral column, generally spanning the region between the T12 and L3 vertebrae ^[28]. Each kidney measures about 11 cm in length, 6 cm in width, and 3 cm in thickness, with an average adult weight of roughly 150 grams ^[29]. The outer layer of the kidney is referred to as the renal cortex, while the inner portion, called the renal medulla, contains the renal pyramids ^[30]. The nephron serves as the kidney's fundamental functional unit and consists of the renal corpuscle together with the renal tubule, carrying out the processes of blood filtration and urine formation ^[31]. Blood is delivered to the kidney through the renal artery, which divides into smaller arterioles, and leaves after filtration through the renal vein ^[32]. The urine produced travels through the collecting ducts into the renal pelvis, continues into the ureter, and is finally stored in the urinary bladder until excretion ^[33].

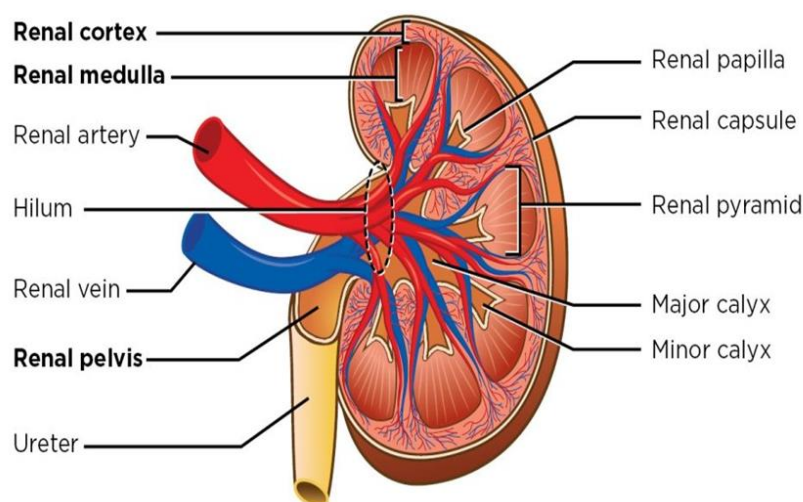


Fig No:1.1 Anatomy of Kidney

The kidney is surrounded by three distinct protective coverings: the renal capsule, which is a dense fibrous membrane directly enveloping the organ; the adipose capsule, providing a cushioning layer of fat; and the renal fascia, which secures the kidney to nearby tissues and structures. Inside the organ, the medulla contains 8–18 cone-like renal pyramids that channel urine first into the minor calyces, then into the major calyces, and ultimately into the renal pelvis. Separating each pyramid are renal columns made of cortical tissue that project inward between them. Each kidney contains roughly one million nephrons, each consisting of a glomerulus enclosed by Bowman's capsule and continuing into the proximal convoluted tubule, the loop of Henle, and the distal convoluted tubule. These components are essential for processes such as ultrafiltration, selective reabsorption, and secretion. Positioned adjacent to the glomerulus is

the juxtaglomerular apparatus, which helps control blood pressure and the rate of filtration by releasing renin when renal perfusion falls. In addition, lymphatic vessels, nerves from the renal plexus, and sympathetic fibers accompany the renal blood supply, working together to regulate kidney activity and vascular tone ^[34].

➤ **Functions of kidney**

The kidneys are vital organs that filter waste products and excess fluids from the blood to form urine. They help maintain the body's fluid and electrolyte balance, regulate blood pressure, and support red blood cell production by releasing a hormone called erythropoietin. The kidneys also help maintain healthy bones by balancing calcium and phosphorus levels.

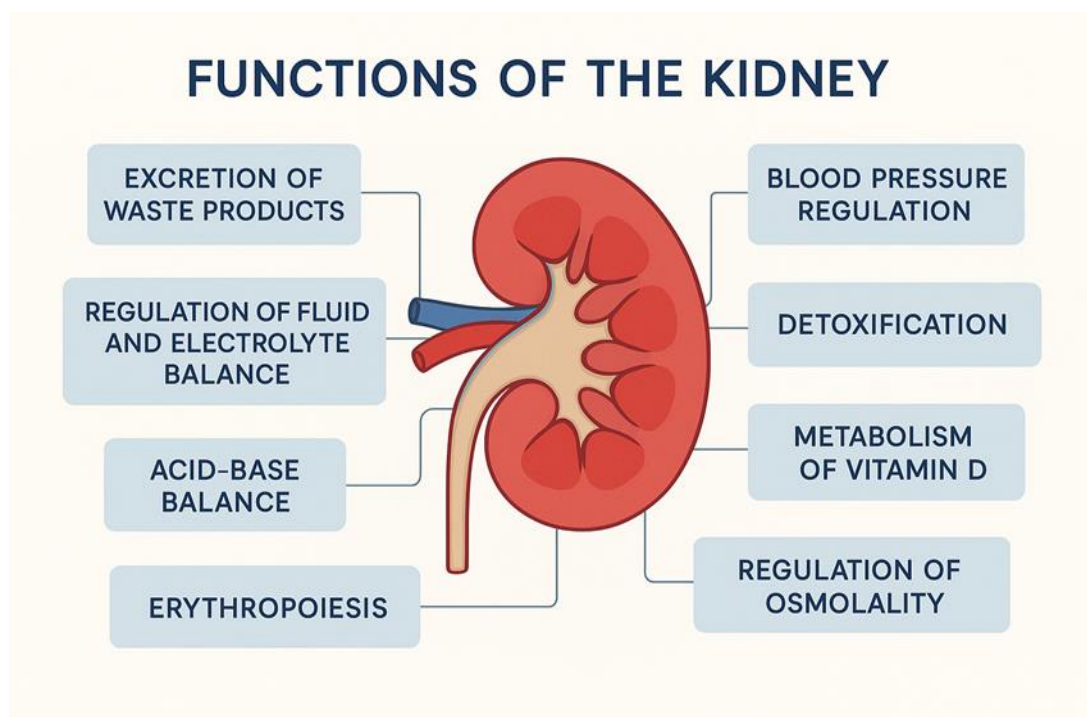


Fig No:1.2 Functions of Kidney

In addition to filtering blood and producing urine, the kidneys play a key role in maintaining the body's internal environment. They help regulate the pH level of the blood, keeping it neither too acidic nor too alkaline. The kidneys also assist in controlling the levels of important minerals such as sodium, potassium, and magnesium. By adjusting the volume and concentration of urine, the kidneys ensure that the body stays properly hydrated and chemically balanced.^[35]

1.1 DIURETICS

Diuretics are therapeutic agents that enhance the elimination of water and electrolytes from the body by stimulating greater urine output in the kidneys. They are frequently prescribed for the treatment of hypertension, heart failure, oedema, and various renal disorders. These drugs function by blocking the reabsorption of sodium at different segments of the nephron, which results in increased excretion of water. Several classes exist, including loop diuretics such as furosemide,

thiazide diuretics such as hydrochlorothiazide, and potassium-sparing diuretics such as spironolactone, each exerting their action at specific locations within the nephron.

1.2 BASIC STEPS IN URINE FORMATION

1. Glomerular Filtration

This process is influenced by the hydrostatic pressure of blood and the selective permeability of the glomerular membrane. Any change in blood pressure or damage to the filtration barrier can significantly affect the efficiency of this step.

2. Tubular Reabsorption

Reabsorption is a highly selective and energy-dependent process that helps maintain the body's water and electrolyte balance. Hormones like aldosterone and parathyroid hormone also regulate the reabsorption of sodium, calcium, and other ions.

3. Tubular Secretion

Tubular secretion acts as a fine-tuning mechanism to eliminate excess ions, toxins, and drugs from the bloodstream. It plays a key role in acid–base regulation by controlling hydrogen ion concentration in the blood.

4. Concentration of Urine

This stage ensures water conservation, especially during dehydration, through the action of ADH. Proper functioning of this mechanism is essential to prevent disorders such as diabetes insipidus or dehydration.

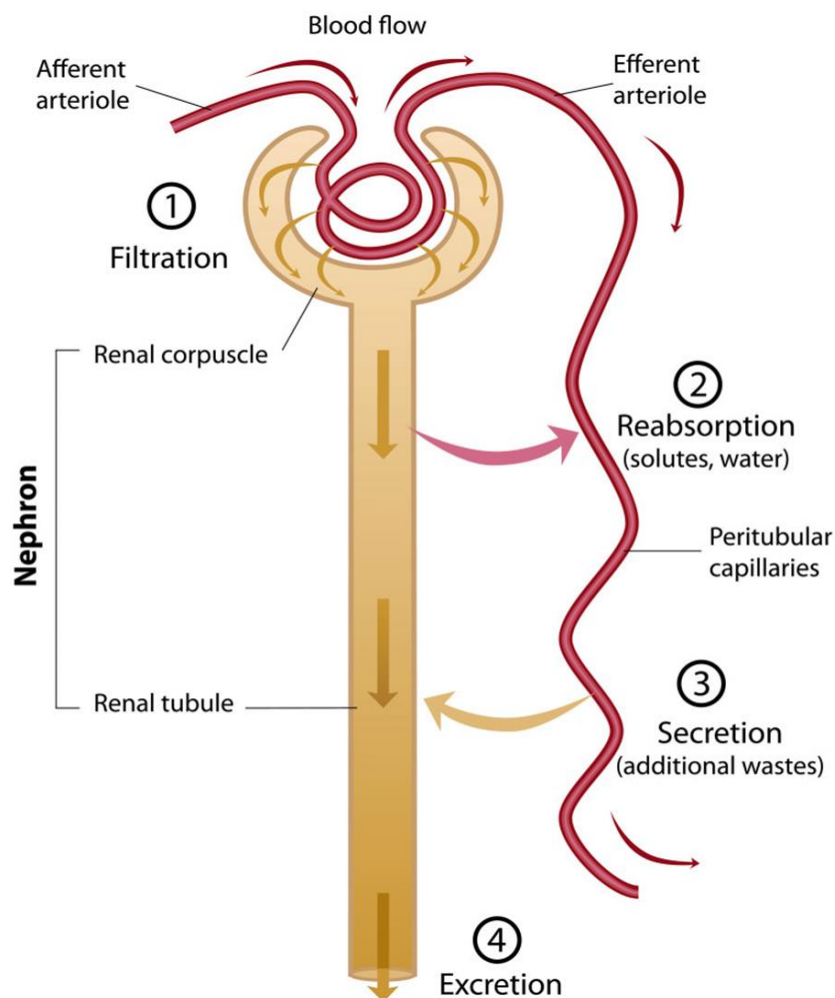


Fig No: 1.3 Steps in urine formation

1.3. THERAPEUTIC AGENTS

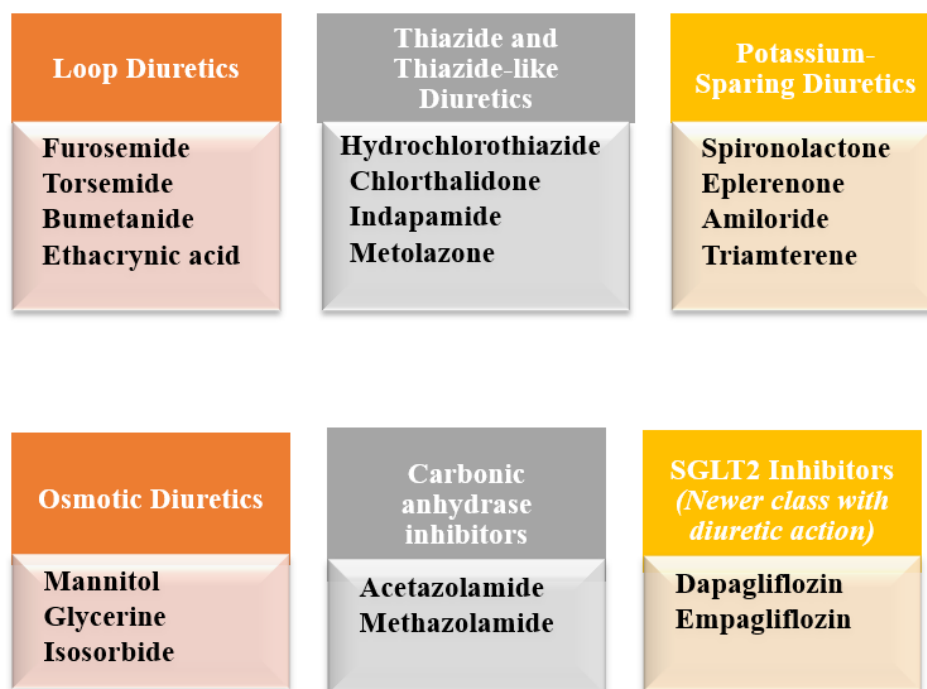


Fig No: 1.4 Classification of Diuretic Drugs

1.4 MECHANISM OF ACTION

Diuretics produce their action by acting on specific portions of the nephron, modifying the balance of sodium, water, and other electrolytes to enhance urine formation. Each category of diuretics possesses a unique mode of action:

➤ Loop Diuretics

Agents such as furosemide, bumetanide, ethacrynic acid, and torsemide operate within the thick ascending limb of the loop of Henle by blocking the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter. This inhibition stops the reabsorption of sodium and consequently increases the excretion of water. Loop diuretics are considered the most powerful type, remaining effective even when the glomerular filtration rate (GFR) is low. Nevertheless, a rapid development of acute tolerance can occur soon after therapy begins, producing what is known as the “braking phenomenon,” in which compensatory mechanisms lead to sodium retention.

Management of this issue may involve modifying the dosage or administration frequency, restricting dietary sodium, or combining a loop diuretic with a thiazide-type diuretic to create a sequential nephron blockade.

➤ Thiazide and Thiazide-like Diuretics

In addition to controlling hypertension, thiazides also reduce calcium excretion, which makes them beneficial in preventing recurrent kidney stones and managing osteoporosis. They are generally well tolerated, but long-term use may still cause mild hypokalemia, hyperuricemia, or impaired glucose tolerance in some patients.

➤ Potassium-Sparing Diuretics

These diuretics are often prescribed in combination with thiazides or loop diuretics to balance potassium levels and minimize the risk of hypokalemia. However, excessive use can lead to hyperkalemia, especially in patients with renal impairment or those taking ACE inhibitors or

ARBs. Their dual role in conserving potassium and managing specific hormonal conditions makes them highly valuable in clinical practice.

➤ Osmotic Diuretics

Although effective, osmotic diuretics must be carefully administered, as excessive use can lead to dehydration, electrolyte imbalance, or worsening heart failure due to rapid fluid shifts. Their rapid onset of action makes them particularly useful in emergency situations where

immediate reduction of intracranial or intraocular pressure is required.

➤ Carbonic Anhydrase Inhibitors

These agents are not commonly used as first-line diuretics due to their weak diuretic effect, but they are highly effective in specialized conditions such as glaucoma, altitude sickness, and certain forms of epilepsy. Their mechanism highlights the close relationship between renal function, electrolyte balance, and respiratory regulation.

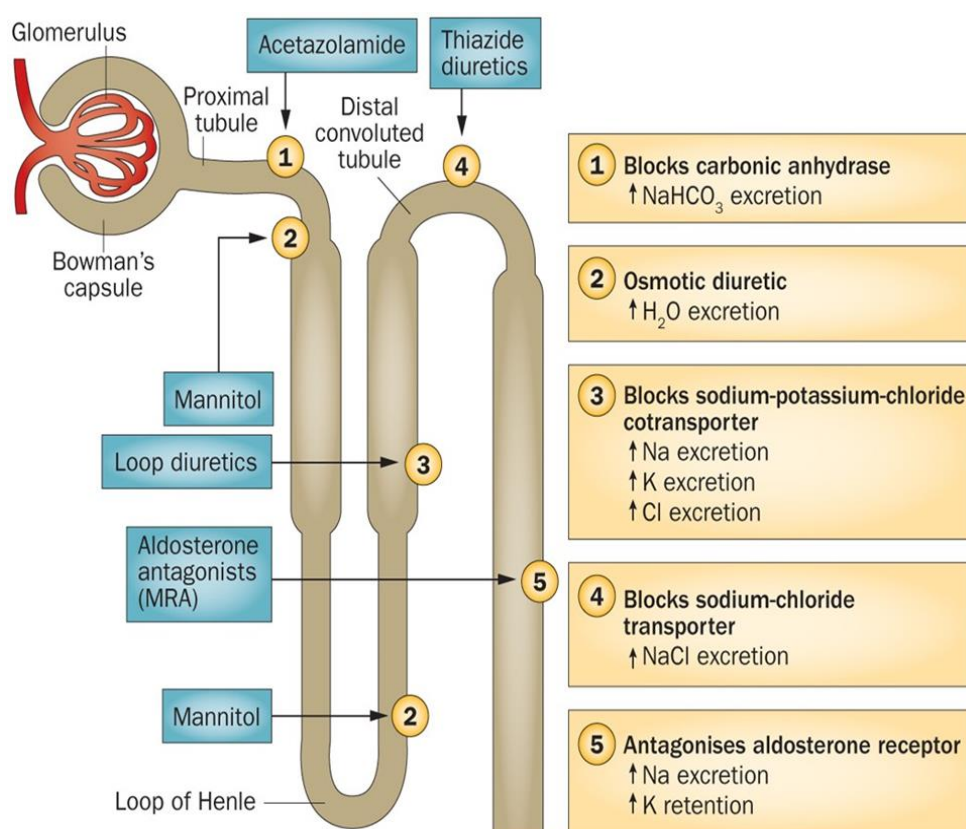


Fig No: 1.5 Mechanism of Action

1.5 EPIDEMIOLOGY

Diuretics are among the most commonly prescribed medications worldwide, especially in the management of hypertension and heart failure. In the United States, data from the National Health and Nutrition Examination Survey (NHANES) show that approximately 15–20% of adults with

hypertension are on diuretic therapy, with thiazide diuretics being the most frequently used class due to their proven benefits in blood pressure control and cardiovascular outcomes.^[37] The prevalence of loop diuretic use is significantly higher among patients with chronic heart failure and advanced kidney disease, reflecting their role in fluid overload management.^[38] Globally, variations in

diuretic use are influenced by regional guidelines, availability, and healthcare infrastructure. For instance, in low- and middle-income countries,

diuretics remain a first-line and cost-effective choice for hypertension management.^[39]

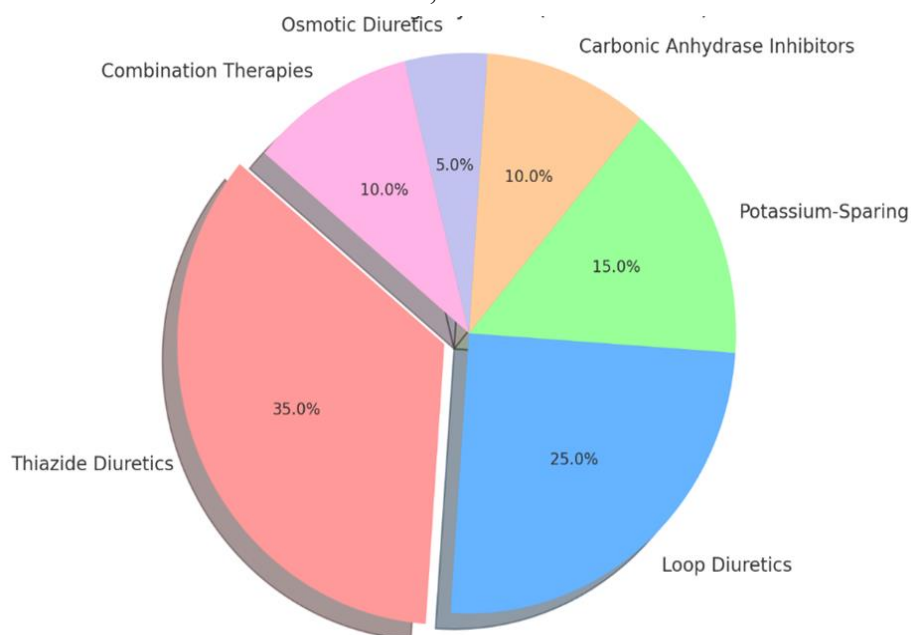


Fig No:1.6 Global diuretic usage by class (2024 estimate)

This pie chart illustrates the estimated global usage of different classes of diuretics in 2024. Thiazide diuretics remain the most commonly prescribed, especially for hypertension, followed by loop diuretics for heart failure and renal conditions.^[40]

1.6 ETIOLOGY

Diuretics are primarily prescribed to treat conditions characterized by fluid retention (oedema) or hypertension.^[41] They are commonly used in congestive heart failure, liver cirrhosis, chronic kidney disease, and nephrotic syndrome, where excess fluid accumulates due to impaired excretion or increased fluid retention mechanisms.^[42] Additionally, diuretics help manage high blood pressure by reducing plasma volume and peripheral vascular resistance.^[43] Their therapeutic use stems from an understanding of renal physiology and electrolyte balance, where modifying sodium and water reabsorption can

alleviate symptoms and prevent complications of volume overload and hypertension.^[44]

1.7 PATHOPHYSIOLOGY

Diuretics function by blocking specific ion transporters or channels, which causes greater excretion of sodium (natriuresis) and water (diuresis) in the urine. This process decreases plasma volume, thereby helping to lower blood pressure and alleviate oedema. Various classes of diuretics act on different regions of the nephron: loop diuretics work on the ascending limb of the loop of Henle; thiazide diuretics act on the distal convoluted tubule; potassium-sparing diuretics influence the collecting duct; and carbonic anhydrase inhibitors act at the proximal tubule^[45]. Through these effects on electrolyte and fluid balance, diuretics alter both hemodynamic and volume status, making them essential for the treatment of conditions such as heart failure, hypertension, and kidney-related disorders.

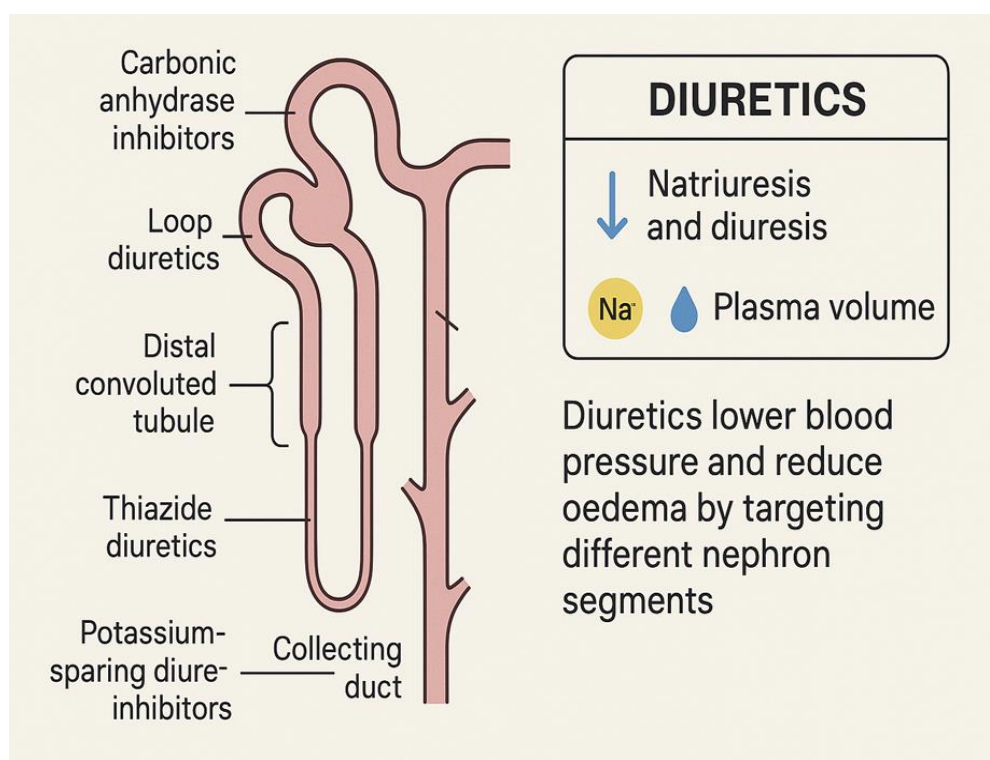


Fig No: 1.7 Pathophysiology of Diuretics

1.8 DIURETICS- SIDE EFFECTS

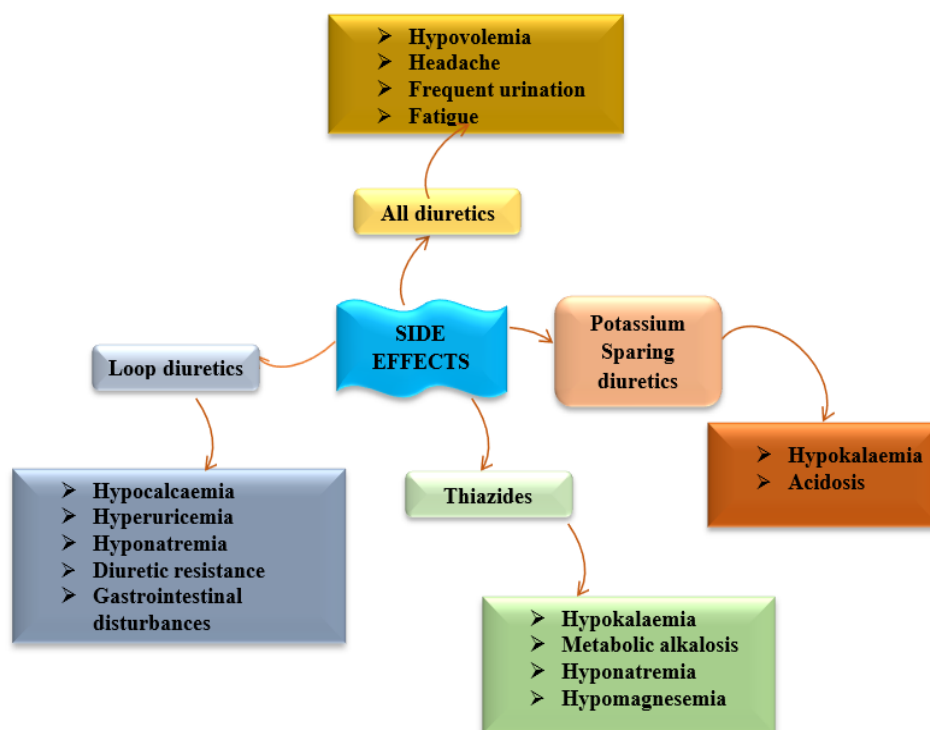


Fig No: 1.8 Side effects of diuretics

1.9 FUROSEMIDE

Furosemide is a powerful loop diuretic commonly prescribed for the treatment of congestive heart failure, oedema, and elevated blood pressure. Its mechanism of action involves blocking the Na^+ - K^+ - 2Cl^- symporter located in the thick ascending limb of the loop of Henle, which promotes the

enhanced excretion of sodium, chloride, and water. Because of its strong diuretic activity, furosemide is particularly valuable in patients who have reduced kidney function. Nevertheless, its use can lead to adverse effects such as hypokalaemia, dehydration, and ototoxicity, especially when administered in high doses or over an extended period [46].

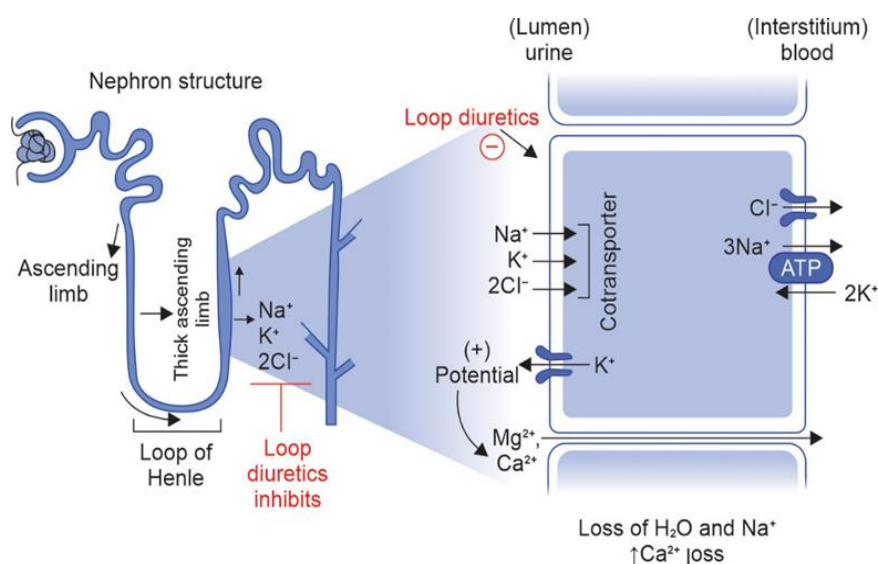


Fig No: 1.9 Mechanism of action of furosemide

➤ Pharmacological action of Furosemide

 Diuretic Effect Inhibition of Na^+-K^+-2Cl^--symporter in the loop of Henle Increased excretion of sodium, chloride, and water	 Antihypertensive Effect Lowers blood pressure Reduces plasma volume
 Venodilation (Acute Effect) Reduces preload Decreases pulmonary congestion	 Electrolyte Imbalance Hypokalemia, hypocalcemia, hypomagnesemia, and hyponatremia

Fig No: 1.10 Pharmacological action of Furosemide

➤ Pharmacokinetics of Furosemide

Furosemide is a loop diuretic with oral bioavailability that typically varies between 50% and 70%, and its absorption can decrease in conditions such as heart failure or oedema. When taken orally, the drug begins to act within about 30 to 60 minutes, whereas intravenous administration produces effects in roughly 5 minutes. It binds extensively to plasma proteins, at about 95%, and has a relatively small volume of distribution of 0.1–0.2 L/kg. Only around 10–20% of furosemide undergoes hepatic metabolism, with most of the dose—approximately 80%—being excreted unchanged by the kidneys through active tubular secretion. In individuals with normal kidney function, the elimination half-life is about 1 to 2

hours, but this period is extended in those with renal impairment. The diuretic effect generally lasts 6 to 8 hours following oral dosing and about 2 to 3 hours when administered intravenously ^[46].

Uses

- Edematous Conditions
- Hypertension
- Acute Pulmonary Oedema
- Hypercalcemia
- Forced Diuresis

1.10 PROPOSED MECHANISM OF ACTION

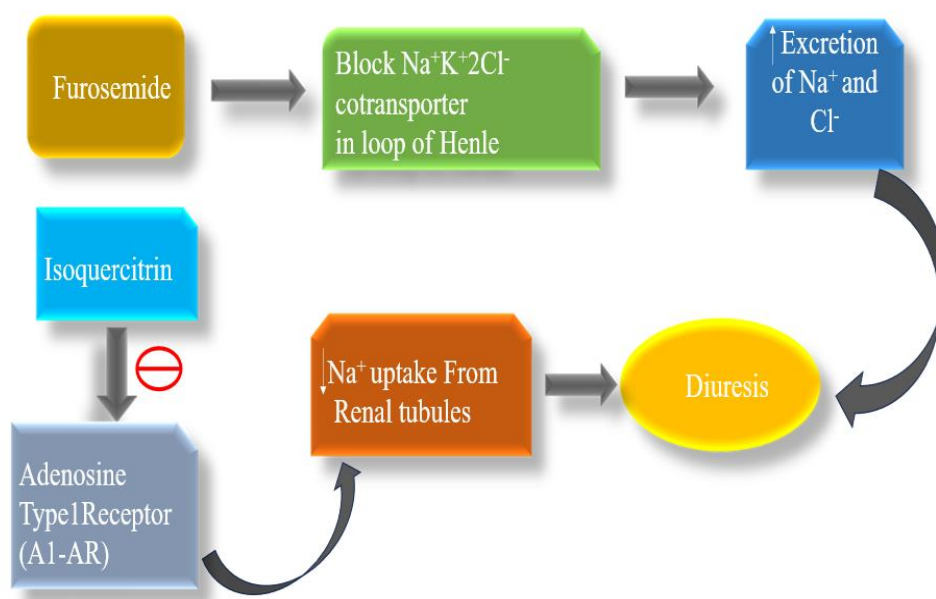


Fig No: 1.11 Proposed Mechanism of action

1. REVIEW OF LITERATURE

Jatmiko Eko Witoyo. et al 2024 ^[47] conducted a detailed evaluation of the phytochemical profile, pharmacological actions, and overall safety of *Morus alba*. They highlighted that the tree contains diverse secondary metabolites such as flavonoids, alkaloids, and phenolic compounds,

which are biosynthesized through multiple pathways. These compounds exhibit remarkable medicinal value, particularly in the management of diabetes, obesity, and neurodegenerative disorders. In addition, phenolic constituents like resveratrol are recognized for their



cardioprotective, anticancer, and anti-aging activities.

Maryam Fatima. et al 2024 ^[48] explored the biosynthesis of bioactive molecules in *Morus alba* and their wide-ranging pharmacological potential. Belonging to the *Morus* genus of the Moraceae family, white mulberry thrives both wild and cultivated in Indonesia. While the fruit remains the most commonly utilized part, the leaves are rich in nutraceutical and therapeutic compounds. These characteristics make the plant highly valuable for applications in both the medicinal and food industries, warranting further scientific investigation.

Namrata Verma. et al 2024 ^[49] reported that the extract of *Morus alba* exhibits strong antioxidant properties, mainly due to its diverse phytochemical constituents such as flavonoids and phenolic compounds. The leaves of white mulberry are enriched with a wide array of bioactive molecules, including alkaloids, anthraquinones, anthocyanins, glycosides, phenolic acids, saponins, steroids, tannins, and others, all of which contribute to significant pharmacological effects. Moreover, the comprehensive nutraceutical composition of these leaves highlights their potential as valuable ingredients for functional foods and therapeutic formulations.

Das R. et al 2024 ^[50] investigated that the use in traditional and modern medicine for treating fever, hypertension, and infections. It also discusses their antimicrobial activity against *E. coli* and *Staphylococcus aureus*, as well as antioxidant activity measured through DPPH assays.

Lee JH. et al 2023 ^[51] investigated that the comprehensive review discusses *M. alba* in relation to metabolic syndrome, particularly focusing on its antidiabetic, antihyperlipidemic,

and anti-obesity activities. The review presents in vitro and in vivo findings supporting its potential in reducing insulin resistance, lowering blood glucose, and modulating lipid profiles.

Chen L. et al 2023 ^[52] investigated that the neuroprotective potential of *M. alba*, particularly its effects on Alzheimer's and Parkinson's disease models. It discusses the modulation of oxidative stress, apoptosis inhibition, and neuroinflammation suppression by mulberry-derived compounds. The study recommends further clinical trials for validation.

Kumar S. et al 2022 ^[53] investigated that the diverse bioactive compounds in *Morus alba*, including flavonoids (such as quercetin, rutin, and isoquercitrin), alkaloids, and tannins. The review highlights its antioxidant, anti-inflammatory, hypoglycaemic, neuroprotective, and hepatoprotective effects. It emphasizes the potential of *M. alba* in managing diabetes, cardiovascular disorders, and neurodegenerative diseases.

Wang Y. et al 2021 ^[54] investigated that the molecular insights into the mechanisms of *Morus alba*'s active compounds. It covers cellular signalling pathways modulated by mulberry constituents, such as the PI3K/Akt pathway in anti-diabetic action and NF-κB inhibition in anti-inflammatory responses. It also highlights emerging roles in cancer prevention and neuroprotection.

Rahman N. et al 2024 ^[55] investigated that the *M. alba*'s neuroprotective mechanisms relevant to Alzheimer's disease (AD) and Parkinson's disease (PD). It outlines how mulberry extracts reduce beta-amyloid aggregation, restore cholinergic transmission, and reduce oxidative neuronal damage via Nrf2/ARE signalling activation. The flavonoid isoquercitrin is identified as a key



compound mediating anti-apoptotic and anti-inflammatory effects. Animal studies cited show improved memory, learning, and motor functions with *M. alba* supplementation.

Singh AK.et al 2023^[56] investigated that it targets *M. alba* in the context of type 2 diabetes mellitus (T2DM). It summarizes studies on the effects of mulberry leaf extract and isolated compounds like 1-deoxynojirimycin (DNJ) in postprandial glucose control. The review also explores the inhibition of intestinal enzymes (alpha-glucosidase and sucrase) and presents clinical trials showing improvements in fasting blood glucose and HbA1c in diabetic patients treated with standardized *M. alba* extract.

Ahmed M.et al 2022^[57] investigated that the clinical and preclinical studies involving *M. alba* leaves. It confirms their hypoglycaemic effect through mechanisms like alpha-glucosidase inhibition, insulin sensitization, and glucose uptake enhancement. It also discusses the leaves' role in reducing LDL cholesterol and improving liver enzyme profiles. Special attention is given to the standardization of extract doses and their compatibility with existing antidiabetic drugs.

Patel D.et al 2021^[58] investigated that the wide-ranging phytochemicals found in *Morus alba*, such as flavonoids, stilbenes, alkaloids, and coumarins. It emphasizes the plant's antioxidant capacity, particularly due to quercetin, rutin, and moracin M. The article thoroughly discusses in vivo and in vitro models demonstrating *M. alba*'s anti-inflammatory, anticancer, antidiabetic, and antimicrobial properties. It also addresses the safety and toxicity profiles, concluding that the leaf and fruit extracts are generally safe for therapeutic use.

Zhao T.et al 2020^[59] investigated that this comprehensive update discusses over 150

compounds isolated from different parts of *M. alba*. The review focuses on moracins, a unique class of stilbenes with antitumor activity, and their ability to induce apoptosis in cancer cells via caspase-3 activation and Bcl-2 downregulation. Additionally, it covers cardioprotective effects, highlighting the plant's ability to modulate angiotensin-converting enzyme (ACE) and prevent vascular inflammation.

Kim MJ.et al 2021^[60] investigated that it integrates both traditional knowledge and modern phytochemistry of *M. alba*. It identifies moracins, flavonols, terpenoids, and fatty acids from different plant parts (leaves, root bark, fruits). It connects these phytochemicals to antiviral, antithrombotic, anti-obesity, and hypolipidemic effects. Special focus is given to in vitro and in vivo evidence supporting these pharmacological roles, making it a resource for preclinical research.

Rajak P.et al 2024^[61] investigated that the meta-review analyses over 200 studies on the phytoconstituents of *M. alba*, focusing on their quantified effects across different extraction methods (aqueous, ethanolic, methanolic). It discusses synergistic actions of flavonoids and alkaloids in conditions like type 2 diabetes, hypertension, and microbial infections. The review also evaluates toxicity studies, finding *M. alba* generally safe at therapeutic doses.

Aher YN.et al 2021^[62] investigated that it provides a comprehensive account of the phytochemicals in *Morus alba*—especially flavonoids (quercetin, rutin), alkaloids, and tannins and their roles in treating diabetes, inflammation, and oxidative stress.

Zhang Y.et al 2023^[63] investigated that the therapeutic roles of *Morus alba* in modern medicine, focusing on metabolic disorders such as diabetes, hyperlipidaemia, and obesity. It



emphasizes the role of active compounds like 1-deoxynojirimycin (DNJ) in inhibiting carbohydrate absorption and improving insulin sensitivity.

Hassan SM.et al 2020^[64] investigated that the lists over 50 herbal diuretics and describes their effectiveness compared to conventional medications. It includes details on plants like *Equisetum arvense*, *Urtica dioica*, and *Hibiscus sabdariffa*, focusing on their renal mechanisms and potential toxicity.

Khan N.et al 2022^[65] investigated that it evaluates traditional herbs for their diuretic activity, emphasizing the role of active phytochemicals such as morin, apigenin, and kaempferol. It explains in detail the methods used for testing in animal models, including the Lipschitz method and measurement of Na⁺/K⁺ excretion.

Almeida N.et al 2023^[66] investigated that the wide range of plant extracts assessed for both diuretic and Sal uretic (salt-excreting) properties. Special attention is given to polyphenolic-rich plants like *Salvia officinalis* and *Rosmarinus officinalis*. It suggests combining pharmacological testing with metabolomic profiling.

Borges J.et al 2021^[67] investigated that the use of natural diuretics in managing fluid overload in heart failure and hypertension. Herbs such as *Taraxacum officinale* and *Petroselinum crispum* are discussed for their potassium-sparing actions and reduced risk of hypokalaemia compared to synthetic diuretics.

Chakraborty A.et al 2023^[68] investigated that it Compares traditional diuretic herbs from Ayurveda, Traditional Chinese Medicine (TCM), and Unani medicine with experimental results from modern pharmacology. Plants like Punarnava, *Gokshura*, and *Zea mays* were

validated for urine output, electrolyte modulation, and kidney safety.

Muthu S.et al 2017^[69] investigated that it evaluates numerous plants traditionally used as diuretics across Asia and Africa. It highlights the role of saponins, tannins, and flavonoids in promoting diuresis, and discusses species like *Boerhaavia diffusa*, *Equisetum arvense*, and *Orthosiphon stamineus*.

Kumar D.et al 2018^[70] investigated that the phytoconstituents of *Morus alba*, including flavonoids (quercetin, rutin), alkaloids, and anthocyanins. It highlights antioxidant, antidiabetic, anti-inflammatory, and neuroprotective activities supported by experimental models.

Andallu B.et al 2016^[71] investigated that the traditional and experimental use of *Morus alba* in managing diabetes, obesity, and hyperlipidaemia. The review discusses active compounds like DNJ (1-deoxynojirimycin) and their inhibitory effects on carbohydrate metabolism enzymes.

Singh R.et al 2017^[72] investigated that the bioactive constituents in *Morus alba* leaves and their effects on various body systems. Reports antimicrobial, antihypertensive, and hepatoprotective activities. Emphasizes its use in traditional Asian medicine.

Rajesh V.et al 2015^[73] investigated that the broad review of *Morus alba*'s Pharmacognostic features, nutritional composition, and bioactivities. Describes its role in treating fever, sore throat, and liver disorders.

Desai SD.et al 2019^[74] investigated that the multipurpose medicinal value of *Morus alba*—including antidiabetic, antioxidant, antitumor, and



anti-obesity effects. Also reviews traditional uses in Chinese, Korean, and Ayurvedic systems.

Thakur M.et al 2019^[75] investigated that the traditional uses of *Morus alba* in Chinese and Ayurvedic medicine, supporting its pharmacological relevance through modern studies. Activities such as hepatoprotective, antimicrobial, and antidiabetic effects are well documented in both in vitro and in vivo models.

Narkhede MB.et al 2016^[76] investigated that it provides an in-depth look at the phytoconstituents of *Morus alba* including phenolic compounds, flavonoids, tannins, and stilbenes. It highlights pharmacological effects such as antidiabetic, antioxidant, neuroprotective, and anti-inflammatory activities, backed by experimental evidence. The authors also discuss its safety profile and potential in formulation development.

2.1 PLANT PROFILE

Plant Name : *Morus alba*

Common Name : Common mulberry, Chinese White mulberry, Silkworm mulberry

Synonyms :

Morus alba var. *constantinopolitana*,

Morus alba var. *multicaulis*,

Morus tatarica,

Morus indica,

Morus multicaulis.

Family: Moraceae

Part Used: Leaves

2.1 ETHNOBOTANICAL SURVEY

VERNACULAR NAMES ^[77]

English - White mulberry

Malayalam - Vellaranchi

Hindi - Shahtoot Safed

Tamil – Vellai Saathukudi

Sanskrit – Tuta

Telugu – Tella Toota

Kannada – Bili Tuti

Marathi – Pandhara Toot

Bengali – Sada Toot

Arabic – Toot

TAXONOMY

Kingdom - Plantae

Class - Magnoliopsida

Subclass – Hamamelididae

Order – Urticales

Family – Moraceae

Genus - *Morus*

Species - *Morus alba*



Fig no: 2.1 *Morus alba* leaves



Fig no: 2.2 *Morus alba* fruit

2.1.1 PLANT DESCRIPTION

Morus alba (MA), often referred to as the white mulberry, is a deciduous tree of moderate size belonging to the Moraceae family. The species generally attains a height of about 10–20 m and develops a comparatively short main stem supporting a broad, spreading canopy. Its leaves are simple, alternately arranged, and exhibit considerable variation in shape; they possess serrated edges and a glossy, bright-green upper surface. The tree bears unisexual blossoms

arranged in catkin clusters, with male and female flowers appearing either on the same individual or on separate plants, indicating a monoecious or dioecious habit. Its fruit is an aggregate type that resembles a blackberry and transitions in colour as it matures, progressing from green to shades of white, pink, or purple when fully ripe.^[78]

The bark of *Morus alba* is greyish-brown with vertical fissures and is often used in traditional medicine. The roots are deep and well-developed, supporting the plant's adaptability to a variety of

soil types and climates. The tree is highly resilient and can tolerate drought, pollution, and heavy pruning, making it suitable for cultivation in both urban and rural areas. Its flowering occurs in spring, and fruiting follows in early summer. The species is widely propagated through seeds, stem cuttings, and grafting techniques for both agricultural and medicinal purposes.^[79]

2.1.2 HABITAT

Morus alba originates in China but has been extensively introduced and naturalized throughout Asia, Europe, Africa, and the Americas. It flourishes in climates ranging from warm temperate to subtropical and shows a preference for well-drained loamy soils, though it can readily adapt to other soil types, including sandy or clay-rich substrates. This hardy species is frequently encountered along roadsides, beside riverbanks, at forest edges, and within cultivated farmland. Because of its strong tolerance to poor conditions, it can establish itself even in degraded soils and is frequently incorporated into agroforestry systems

and reforestation programs. The tree also endures periods of drought and is capable of surviving in regions with limited rainfall.^[80]

2.1.3 NUTRIENT COMPOSITION

Various parts of *Morus alba*, including its leaves and fruits, are abundant in nutrients and biologically active compounds. The leaves are particularly recognized for their elevated protein levels, ranging from 20 to 27% of dry weight, which makes them an important feed source for silkworms as well as livestock. Additionally, the leaves provide essential amino acids, vitamins such as C, A, and B-complex, along with minerals including calcium, potassium, magnesium, iron, and zinc. The fruits of *Morus alba* are low in calories but rich in dietary fiber, natural sugars (primarily glucose and fructose), antioxidants like flavonoids and anthocyanins, and polyphenolic compounds, all of which play a role in promoting health.^[81]

2.1.4 CHEMICAL CONSTITUENTS

Table no:2.1 Chemical constituents of *Morus alba*

Plant Part	Chemical Constituents
Leaves	Rutin, Isoquercitrin, Quercetin, Chlorogenic acid, β -sitosterol
Fruits	Anthocyanins, Resveratrol, Vitamin C, Glucose, Fructose
Root Bark	Morusin, Kuwanon C, Oxyresveratrol, Cyclomulberrin
Stem Bark	Scopoletin, Mulberroside A, Tannins
Seeds	Fatty acids (linoleic, oleic), Proteins
Whole Plant	Alkaloids, Terpenoids, Saponins, Tannins, Steroids

2.1.5 USES

Table no:2.2 Uses of *Morus alba*

Category	Part Used	Use/Activity
Traditional	Leaves, Bark	Antidiabetic, anti-inflammatory
Medicine	Roots	hepatoprotective, diuretic
Nutritional	Fruits, Leaves	Edible fruits (rich in vitamin C, Fiber), leaf tea, animal fodder
Pharmacological	Leaves, Bark, Roots	Antioxidant, neuroprotective, anti-obesity, anticancer
Cosmetic	Leaves, Bark	Used in skin-whitening and anti-aging products



Industrial	Leaves	Primary feed for silkworms in sericulture
Woodwork	Wood (Trunk/Stem)	Used in making tools, furniture, and handicrafts

2.1.6 PREPARED HERBARIUM OF MA



Fig no: 2.3 Herbarium of MA Plant

3. AIM AND OBJECTIVE

3.1 AIM

To evaluate the diuretic activity of ethanolic leaf extract of *Morus alba* in Albino rat.

3.2 OBJECTIVES

- To prepare the ethanolic leaf extract of *Morus alba* by Soxhlet extraction method.
- To evaluate the phytochemical constituents of the crude extract.

- To Perform docking studies.
- To evaluate the diuretic activity by *in vitro* model.
- To evaluate the diuretic activity of ethanolic leaf extract of *Morus alba* by *in vivo* model.
- To compare the effect of ethanolic leaf extract of *Morus alba* with standard drug furosemide.

3.3 PLAN OF WORK

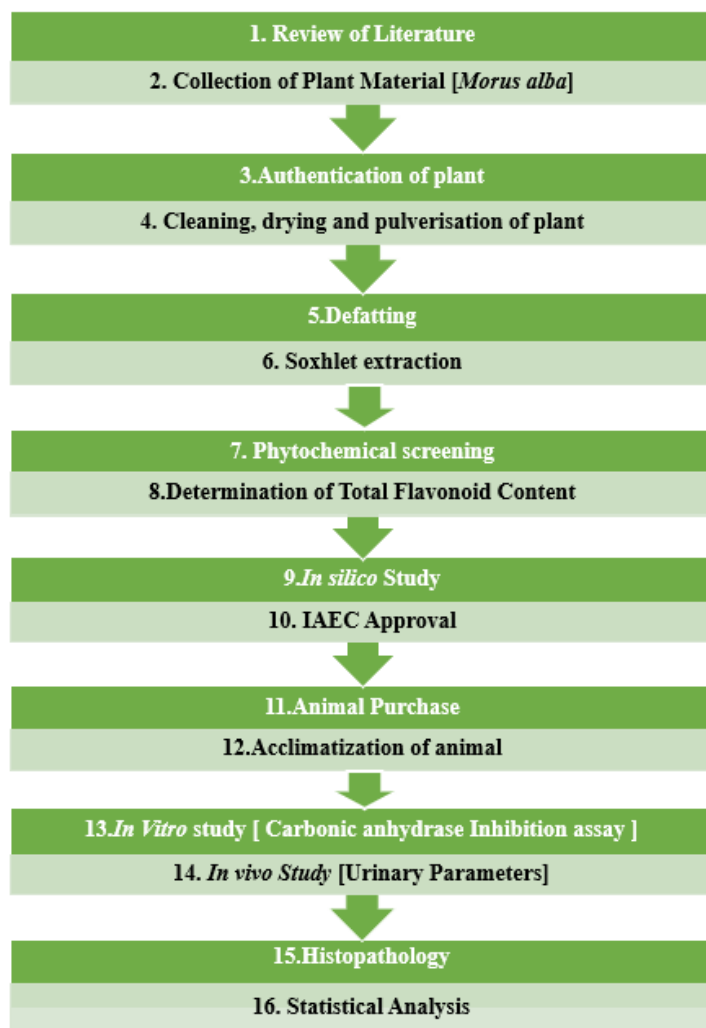


Fig no: 3.3 Diagrammatic representation of plan of work

4. MATERIALS AND METHODS

4.1.1 Plant material

4.1 MATERIALS

Table no:4.1 Description of Plant Material

Sl No.	Plant Name	Plant Used	Family	Collection Period	Collection Point
1	<i>Morus alba</i>	Leaves	Moraceae	February- March	Parassala

Table no:4.2 Ingredients Used for Study

Sl.No	Product	Manufacture
1.	Furosemide	Yarrow Chem chemicals, Mumbai
2.	Ethanol	Sree Krishna College of Pharmacy and Research centre, Parassala
3.	Petroleum Ether	Associated Scientific Company, Thiruvananthapuram
4.	Bedding Materials	Dream Digital, Dhanuvachapuram, Neyyatinkara

5.	Animal Feed	Cape Bio Lab and Research Centre, Marthandam, Tamil Nadu
6.	Oral Feeding Tube	Sree Krishna College of Pharmacy and Research centre, Parassala
7.	Male <i>Wistar albino rat</i>	Cape Bio Lab and Research Centre, Marthandam, Tamil Nadu

4.1.2 Animal used for study

Male Wistar albino rats aged six to eight weeks and weighing between 150 and 200 g were procured from Cape Bio Lab and Research Centre, Marthandam, Tamil Nadu. The animals were housed in clean conditions under a 12-hour light/dark schedule and allowed to acclimatize for seven days at a controlled temperature of 25 ± 2 °C with relative humidity maintained at 40–60%. They were given free access to drinking water and a standard pellet diet throughout the study. The experimental protocol received approval from the Institutional Animal Ethics Committee (IAEC) on February 1, 2025 (approval number: [01/02/IAEC/SKCPRC/2025]) and was carried out in compliance with the regulations of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), New Delhi, India.

4.2 METHODS

4.2.1 Gathering and authentication of *Morus alba*

In February and March, *Morus alba* leaves were gathered from Parassala, Thiruvananthapuram and authenticated by the Department of Botany at Nesamony Memorial Christian College, Marthandam.

4.2.2 Preparation of ethanolic leaf extract of *Morus alba*

Fresh leaves of *Morus alba* were collected and thoroughly rinsed with distilled water to eliminate

dust and other impurities, then left to dry in the shade at room temperature for 7–10 days. Once dried, the leaves were finely powdered using an electric grinder and stored in airtight containers. About 100 g of this powdered material was placed in a thimble and extracted in a Soxhlet apparatus. To remove chlorophyll, waxes, and lipids, the sample was first defatted with petroleum ether for 6–8 hours. The defatted residue was subsequently subjected to extraction with 70% ethanol for approximately 8–12 hours, maintaining a temperature of 50–60 °C. During this process, the ethanol solvent repeatedly refluxed through the plant material, dissolving the bioactive constituents and collecting them in the extraction flask. After completion, the solvent was concentrated under reduced pressure with a rotary evaporator to yield a crude extract, which was then stored in an airtight container at 4 °C until further investigation [82].

4.2.3 Soxhlet extraction method

1. The Soxhlet apparatus was cleaned thoroughly and rinsed with petroleum ether.
2. The defatting of the plant sample was carried out in the Soxhlet apparatus using petroleum ether as the solvent, which removed non-polar lipids and other fat-soluble impurities from the material.
3. The defatted material was dried before starting the extraction.
4. The apparatus was washed again and rinsed with ethanol.



5. The Soxhlet setup was assembled, consisting of a heating mantle to provide controlled heat, a round-bottom flask to hold the extraction solvent, an extraction chamber containing the plant material, a condenser at the top to condense vaporized solvent, and rubber tubing to circulate cooling water through the condenser
6. Ethanol was used to rinse the apparatus before use.
7. Approximately 100 g of air-dried and finely powdered *Morus alba* leaves were accurately weighed and placed into a thimble, which was then inserted into the Soxhlet extraction chamber.
8. The round-bottom flask was positioned on the heating mantle.
9. The extraction chamber was placed above the flask, and the condenser was attached to the top of the chamber.
10. The condenser had an inlet and outlet connected to rubber tubing for water circulation to maintain temperature.
11. About 1000 ml of ethanol was poured into the condenser, which filled the round-bottom flask and circulated through the Soxhlet system to extract the desired compounds from the plant material.
12. The extraction process was started and maintained at 50–60 °C.
13. After completion of the extraction, the ethanol was removed from the extract by evaporation, typically using a rotary evaporator or by gentle heating under reduced pressure, until a dry extract remained.
14. The resulting dried extract was carefully weighed to determine its mass, and the percentage yield was calculated relative to the initial weight of the plant material to assess the efficiency of the extraction. ^[83,84]

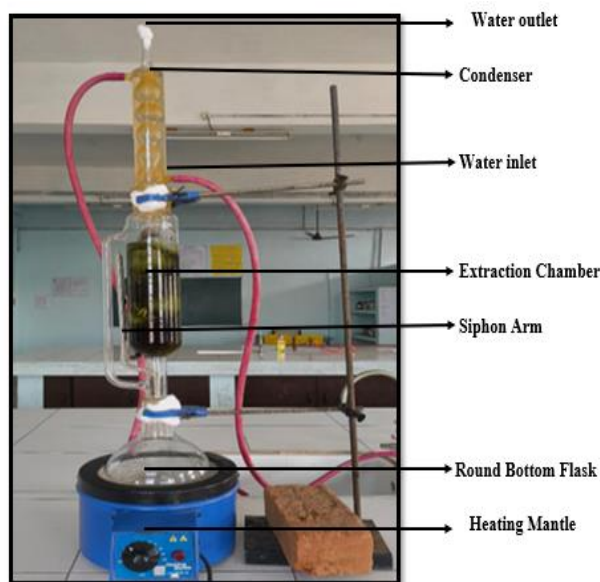


Fig No:4.1- Soxhlet extraction process

4.2.3.1 Computation of output efficiency

The extraction yield and the content of major compounds in the extract were calculated relative

to the weight of the initial crude plant material using the following formula.

$$\text{Percentage yield} = \frac{W_2}{W_1} \times 100$$

Where, W₁- Weight in grams of dried leaves powder of *Morus alba*.

W₂- Weight in grams of extracts obtained

4.2.4 Preliminary phytochemical screening of crude extract of *morus alba*

The crude extracts were first subjected to phytochemical screening to determine the presence of various bioactive constituents. A qualitative approach was employed to detect and characterize these compounds. The chemical profile was deduced based on the relative abundance of the compounds present in the extract. The pharmacological effects of the extract are primarily linked to its phytochemical constituents. Standard qualitative chemical assays were performed according to established protocols to assess the phytochemical composition of the extract. The analysis demonstrated the presence of key secondary metabolites, including flavonoids, tannins, saponins, and phenolic compounds, which are recognized for their contribution to the medicinal potential of the plant.

4.2.4.1 Detection of Flavonoids

a) Alkali Test

The Sample solution was treated with increasing amount of sodium hydroxide and observed for the formation of yellow colour.

b) Lead acetate test

Mixed 2 ml extract with 1 ml of lead acetate solution and observed yellow colour precipitate.

c) Shinoda test

Added Magnesium powder and few drops of concentrated hydrochloric acid or sulphuric acid to 2 ml of extract solution and observed orange colour.

4.2.4.2 Detection of Phenols

a) Lead acetate test

Mixed 2 ml of the extract with 1 ml of lead acetate solution and observed for the formation of white precipitate.

4.4.4.3 Detection of Tannins

a) Ferric chloride test

Mixed 2 ml of the extract with few ml of 5% ferric chloride solution and observed green black colour.

4.4.4.4 Detection of Saponins

a) Foam test

Shake few ml of extract with 5 ml of distilled water and then observed for the formation of form.

4.4.4.5 Detection of Alkaloid

a) Hager's test

2ml of extract was treated with 1-2ml of Hager's reagent (Saturated picric acid solution) and observed yellow colour.

b) Dragendorff's test

2ml of the extract was treated with 1-2ml of Dragendorff's reagent (Potassium bismuth iodide) and then observed reddish brown precipitate.

4.4.4.6 Detection of Terpenoids

a) Salkowski test



Dissolved 1-2ml of sample in 1 ml of chloroform and added 1ml of conc. Sulphuric acid and observed red colour chloroform layer.^[85]

4.2.4.7 Determination of total flavonoid content

Principle

The total flavonoid content was determined using the aluminium chloride colorimetric assay. The fundamental principle of this method is that aluminium chloride interacts to form acid-stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group present in flavones and flavonols. Additionally, it can form acid-labile complexes with ortho-dihydroxyl groups located on the A- or B-ring of flavonoid molecules.

Procedure

The total flavonoid content was determined using the aluminium chloride colorimetric assay. An aliquot of the extract (1 mg/ml) was transferred into a test tube containing 3 ml of distilled water. Then, 250 µl of 5% NaNO₂ was added, and after 5 minutes, 500 µl of 10% AlCl₃ was introduced, followed by 2 ml of 1 M NaOH at the sixth minute. The total volume was adjusted to 10 ml with distilled water to prepare the sample solution. A series of quercetin standard solutions (10, 20, 40, 60, 80, and 100 µg/ml) were prepared in the same manner as described above. The mixture was thoroughly mixed, and the absorbance of the resulting pink-colored solution was measured at 510 nm against a reagent blank. The total flavonoid content was expressed as µg of quercetin equivalents (QE) per mg of extract^[86]

4.2.5 DOCKING STUDIES

Principle

MZDock is an in silico molecular docking tool designed for protein–protein docking, particularly

for symmetric multimers. It predicts how two or more protein molecules interact and form a stable complex. The principle is based on rigid-body docking, where the input structures are treated as fixed, and the algorithm searches for the best orientations and positions that minimize the interaction energy. MZDock uses a fast Fourier transform (FFT) approach to efficiently explore rotational and translational space and score configurations based on shape complementarity and electrostatics.

1. Ligand Preparation

- Input: Ligands can be provided in various formats such as SMILES, PDB, Mol, Mol2, or SDF.
- Energy Minimization: The ligands are then subjected to energy minimization using a chosen force field (MMFF94, MMFF94s, UFF, GAFF, or chemical) and the steepest descent algorithm.
- Protonation States: The protonation state of the ligands can be specified.
- Enantiomer Generation: For SMILES input, MzDOCK offers the option to generate enantiomers.
- Output: Batch scripts convert the energetically optimized ligands into PDB format for docking.

2. Receptor Preparation

- Input: The target protein structure is provided in PDB format.
- Refinement: MzDOCK allows refining the structure by correcting bond orders and adding hydrogen atoms.



- Options for Refinement: Users can add or remove hydrogens, modify partial charges (Kollman or Gasteiger), delete or keep heteroatoms, and retain ions, cofactors, or even crystallographic water molecules.
- Tool: MzDOCK utilizes the AD4 Receptor Preparation tool from Auto Dock Tools for receptor preparation.
- Output: The prepared receptor is saved as a pdbqt file.

3. Binding Site Definition

- The binding site can be defined through manual selection, centring around a co-crystallized ligand or other non-protein elements in the pdbqt file, or by centring the grid box around a specific atom.
- A grid box with adjustable size (up to 20 Å with a buffer) is generated.
- MzDOCK also supports flexible docking by allowing for side-chain flexibility of binding site residues, which can be automatically selected or manually chosen.

4. Molecular Docking Simulation

Molecular docking is performed using the Smina algorithm, generating a user-specified number of top-ranked poses per ligand. The output includes pdbqt files of docked complexes and .log files with binding scores. For flexible docking, an additional pdbqt file of the rearranged binding site is created.

5. Analysis of Results

The PLIP package analyzes interactions between binding site residues and docked ligands. Output files include pdbqt files, a Report.txt detailing

interactions, a PyMOL session file for visualization, and PNG images. While MzDOCK lacks its own analysis platform, pdbqt files can be visualized using external software like PyMOL to examine interactions.

4.2.6 *IN VITRO* DIURETIC ACTIVITY

Carbonic anhydrase inhibition method

Acetazolamide is among the earliest synthetic non-mercurial diuretics and exerts its effect by inhibiting the enzyme carbonic anhydrase. This zinc-containing enzyme catalyzes the reversible conversion of carbon dioxide (CO₂) into carbonic acid (H₂CO₃). Subsequently, carbonic acid dissociates into bicarbonate ions (HCO₃⁻) and hydrogen ions (H⁺) as part of this reaction.

Procedure

Spectrophotometric analysis was conducted using 20 mM HEPES–Tris buffer (pH 7.4) at 25 °C. In each reaction tube, 1,400 µL of HEPES–Tris buffer was mixed with 400 µL of carbonic anhydrase enzyme solution (0.1 mg/mL in the same buffer) and 400 µL of the test compound dissolved in HPLC-grade DMSO, ensuring that the solvent made up 10% of the total volume. This mixture was pre-incubated for 15 minutes at 25 °C. The substrate, p-nitrophenyl acetate (0.7 mM), prepared in HPLC-grade methanol, was added (400 µL) to initiate the reaction. Product formation was monitored at 400 nm using an Elico UV spectrophotometer (India). Enzyme activity in the control was considered 100%, and all experiments were carried out in triplicate for each concentration, with the results reported as the mean of these replicates.

4.2.7 ACCLIMATIZATION OF ANIMALS

Adult male albino rats weighing 150–200 g were acclimated for seven days in the animal facility of



Sree Krishna College of Pharmacy and Research Centre under standard laboratory conditions. The animals were kept in cages fitted with stainless steel grid tops and durable plastic sides. They had unrestricted access to fresh drinking water and a regular pellet diet. The animal room was maintained at a constant temperature of $22 \pm 2^\circ\text{C}$ with a relative humidity of 45–60% and a 12-hour light/dark cycle. Daily monitoring was carried out to confirm their good health and proper adjustment to the experimental setting.

4.2.8 IN VIVO DIURETIC ACTIVITY

4.2.8.1 EXPERIMENTAL DESIGNING

Animal: Male Albino Rat

Age and weight: Six to eight weeks old, 150-200g

Total number of animals needed: 24 Nos

Total number of groups: 4 groups

Number of animals in each group : 6 animals

Table no:4.3 Experimental designing (Grouping)

Name of group	Drug to be administer	Number of animals
Group I– Control group	Normal saline 10 mL/kg orally ^[87]	06
Group II– Standard group	Furosemide 10mg/kg orally ^[87]	06
Group III– Test group I	Ethanollic extract <i>Morus alba</i> 200mg/kg ^[88,89]	06
Group IV– Test group II	Ethanollic extract <i>Morus alba</i> 400mg/kg ^[88,89]	06

4.2.8.2 DIURETIC ACTIVITY IN RAT [LIPSCHITZ MODEL]

Adult male albino rats weighing between 150 and 200 g were randomly divided into four groups, with six animals in each group. The study was

performed at room temperature ($25 \pm 2^\circ\text{C}$). During the experimental period, the animals were not provided with food or water. Group I served as the control and received 10 mL/kg of normal saline orally (equivalent to 1.5–2 mL per rat). Group II, the standard treatment group, was given furosemide at a dose of 10 mg/kg (1.5–2 mg per rat). Groups III and IV, designated as the test groups, received different doses of the ethanolic leaf extract of *Morus alba* (EEMA). All treatments were prepared in the same volume of normal saline to ensure uniform liquid administration across animals. Immediately following administration, each rat was placed in an individual metabolic cage designed to separate urine from feces. Urine was collected in graduated vials over a 6-hour period, measured, and expressed as milliliters per 100 g of body weight.^[88]

4.2.8.3 PARAMETERS EVALUATED

1. Urinary parameters

- Measurement of sodium (Na^+), potassium (K^+), and chloride (Cl^-) ion concentrations in freshly collected urine samples.
- Determination of the pH of freshly collected urine samples.

2. Calculation of Diuretic index, Lipschitz value, Saluretic index and Na^+ / K^+ ratio

$$\text{Diuretic index} = \frac{\text{Mean urine volume of the test group}}{\text{Mean urine volume of the control group}}$$

$$\text{Lipschitz value} = \frac{\text{Mean Urine volume of the test group}}{\text{Mean urine volume of the reference group}}$$

$$\text{Saluretic index} = \frac{\text{Concentration of electrolyte in urine of the test group}}{\text{Concentration of electrolyte in urine of control group}}$$



$$\text{Na}^+ / \text{K}^+ \text{ ratio} = \frac{\text{Concentration of Na}^+ \text{ in urine of test group}}{\text{Concentration of K}^+ \text{ in urine of standard group}}$$

4.2.8.4 HISTOPATHOLOGICAL INVESTIGATIONS

Procedure

Fixation

- 1) Take sections of the rat organs.
- 2) Place the section inside a capsule.
- 3) Fix it in the capsule using formalin (10%) to prevent the decaying of the tissue.
- 4) Wash the capsule in a bowl of water.

Tissue Processing

- 1) After washing the capsule is dipped in absolute acetone for half an hour, then in 90% acetone for next half an hour, then in 80% acetone and 70 % acetone, half an hour each.
- 2) Then it is dipped in concentrated xylene 1st for half an hour and Xylene 2nd another half an hour.
- 3) Wipe and dry the capsule using a cloth.
- 4) Place it in hot paraffin wax in the incubator which helps in removing the xylene content.
- 5) Again, place the capsule in paraffin wax '2nd' to remove the entire xylene content for 3-5 hours.
- 6) The tissue is separated from the capsule.
- 7) Using 'L-block' hot paraffin wax is poured inside it and the tissue is placed inside the wax.

- 8) Cool it for 10 minutes and cut the wax into perfect cubes leaving the tissue untouched.
- 9) Fix the wax cube over the extended part of the block holder exposing the waxy part.
- 10) The block holder is fitted to an instrument called Rotary Microtome where a disposable knife in it trims the wax off leaving the tissue with very little amount of wax.
- 11) The left-out wax is then melted when the tissue is placed in a water bath.

Slide preparation & staining

- 1) Take a glass slide and spread egg albumin (mixture of equal amount of egg white and glycerine) on it so the tissue gets fixed properly.
- 2) Place the section over it and incubate. It for 15 minutes. Then cool it.
- 3) Then the slide is dipped in xylene for 10 minutes. isopropyl alcohol 5 minutes. Then wash running water for 15 minutes. Then Harris Haematoxylin for 5-8 minutes. Then wash it water. Then dip acid alcohol. Then wash water for 10-15 minutes.
- 4) It is then dipped in eosin 3 min. and absolute alcohol 2min and dry properly.
- 5) Finally, it is dipped in xylene for 10 minutes to make the tissue clean.
- 6) DPx (a glue-like substance) is spread over the coverslip and the glass slide is placed in such a way fixing the tissue on the DPX.
- 7) Using forceps the coverslip is fixed properly without creating air bubbles.
- 8) Then the slide is diagnosed using microscope.



4.2.8.5 STATISTICAL ANALYSIS

The data was statistically evaluated using GraphPad Prism software (version 10.3.1). A one-way analysis of variance (ANOVA) was used to compare the outcomes in order to evaluate the differences between the experimental groups^[90].

5. RESULTS

5.1 *Morus alba*: Preparation of ethanolic leaf and the calculation of percentage yield.

Morus alba leaves were gathered from Parassala. Soxhlet extraction was used to successfully extract

the powdered leaf components. After that, the extracts were kept in an airtight, tightly sealed container until they were needed again. Table No. 5.1 displays the output efficiency of the *Morus alba* leaf extract.

Table no 5.1 Percentage yield of leaf extract of *Morus alba*

Plant name	Parts used	Methods of extraction	Solvent used	Percentage yield(%W/W)
<i>Morus alba</i>	Leaves	Soxhlet Extraction Method	Ethanol	20.76 %w/w



Fig No: 5.1 *Morus alba* Ethanolic extract

5.2 Primary phytochemical assessment of ethanolic extract of *morus alba*.

The ethanolic leaf extract was filtered to remove the *Morus alba* phytochemical additions. To determine the phytochemical contents, a variety of chemical assays are utilized, such as the Salkowski, Dragendroff, Hagers, and Alkali tests, as well as the Shinoda, Ferric chloride, Foam, and Alkali tests. Flavonoids, phenols, tannins,

saponins, alkaloids, and terpenoids, in that order, were validated by the findings of these tests. Conventional methods have been used to perform a qualitative evaluation of the extract in order to ascertain its phytochemical components.

5.2.1 Detection of Flavonoids

a) Alkali Test

In Alkali test, Flavonoids were indicated by yellow color.



Fig No: 5.2 Result of Alkali test

b) Lead Acetate Test

In Lead Acetate test, the presence of flavonoids was shown by a yellow precipitate.



Fig No: 5.3 Result of Lead Acetate test

d) Shinoda test

In Shinoda test, the presence of flavonoids was indicated by the orange color.

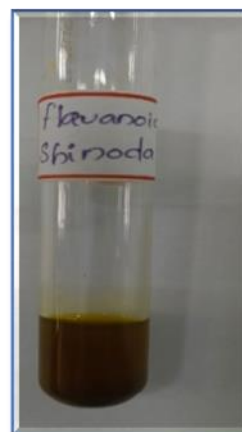


Fig No: 5.4 Result of Shinoda test

5.2.2 Detection of Phenols

a) Lead acetate test

In Lead Acetate test, the presence of phenols was revealed by white precipitate.



Fig No: 5.5 Result of Lead Acetate test

5.2.3 Detection of Tannins

a) Ferric chloride test

In Ferric Chloride Test, Tannins were signified by the colour green-black.

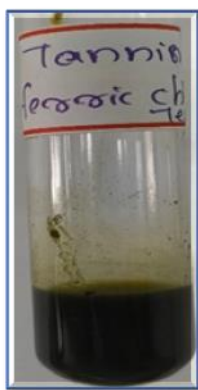


Fig No: 5.6 Result of Ferric Chloride test



Fig No: 5.8 Result of Hager's test

5.2.4 Detection of Saponins

a) Foam test

In Foam test, no formation of foam indicated the absence of Saponins.



Fig No: 5.7 Result of Foam test

b) Dragendorff's test

In Dragendorff's test, absence of reddish-brown precipitate formation suggested that alkaloids were not detected.



Fig No: 5.9 Result of Dragendorff's test

5.2.5 Detection of Alkaloid

a) Hager's test

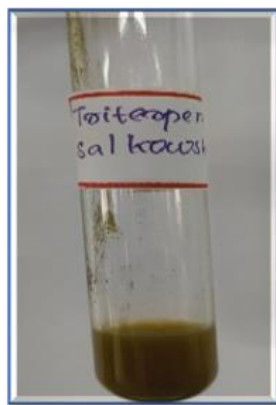
In Hager's test, no Formation of yellow colour indicated the absence of Alkaloids.

5.2.6 Detection of Terpenoids

a) Salkowski Test

In Salkowski Test, no formation red colour of chloroform layer indicated the absence of Terpenoids.

Fig No: 5.10 Result of Salkowski Test

Table No. 5.2 Preliminary phytochemical screening of leaf extract of *Morus alba*

Sl. No	Phytochemical Constituents	Chemical Test	Ethanollic Extract of <i>Morus alba</i>
1	Flavonoids	Alkali Test	+
		Lead Acetate Test	+
		Shinoda Test	+
2	Phenols	Lead Acetate Test	+
3	Tannins	Ferric Chloride Test	+
4	Saponins	Foam Test	-
5	Alkaloids	Dragendroff's Test	-
		Hager's test	-
6	Terpenoids	Salkowski Test	-

(+) Positive

(-) Negative

5.2.7 DETERMINATION OF TOTAL FLAVONOID CONTENT

The current investigation of the ethanolic extract of *Morus alba* (EEMA) demonstrated the presence of most of the secondary metabolites examined, as summarized in Table 5.4. Total flavonoid content was quantified using a standard quercetin calibration curve ($y = 0.0079x - 0.0063$, $R^2 = 0.9992$) (Fig. 5.11). The extract was found to contain 42.82 mg/g of flavonoids, expressed as quercetin equivalents, which aligned with the results of the qualitative analysis. This comparatively high flavonoid content (42.82 mg/g) is noteworthy. Consequently, the flavonoid isoquercitrin appears to be present in considerable amounts in *Morus alba*.

Table No. 5.3 Standard Quercetin

Standards	Concentration of Quercetin ($\mu\text{g/ml}$)	Absorbance at 510 nm
S1	10	0.074
S2	20	0.155
S3	40	0.302
S4	60	0.458
S5	80	0.629

Table No: 5.4 Total amount of Flavonoid content of *Morus alba*

Sample code	Absorbance at 510nm	Concentration of Flavonoid (mg/g)
MA	0.332	42.82

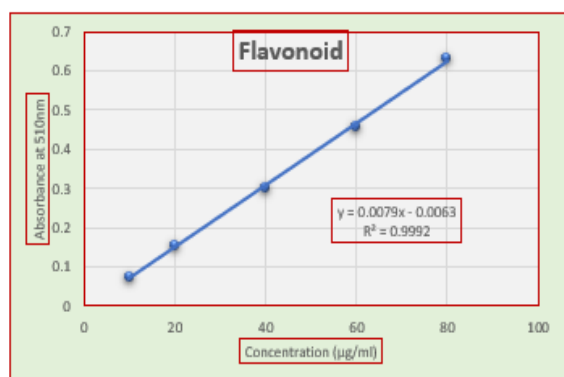


Fig No:5.11- Standard calibration curve of quercetin for total flavonoid content

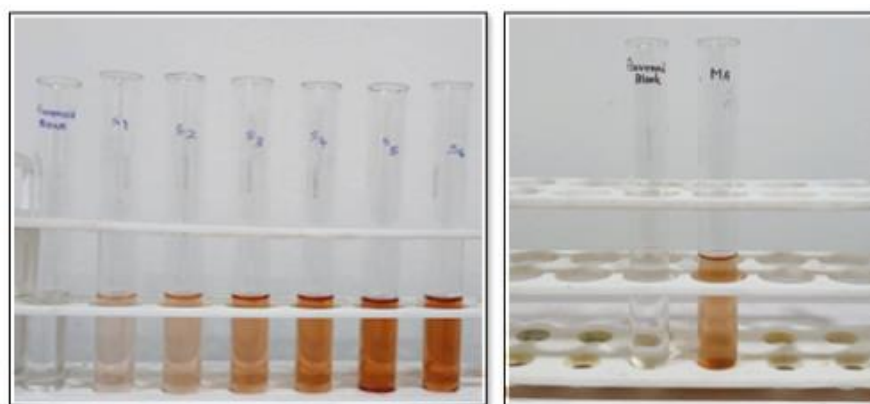


Fig No: 5.12-Results of total Flavonoid Content

5.3 DOCKING STUDIES

Docking studies and binding affinity scores were performed for each identified phytochemical from *Morus alba*. The most reliable receptor-ligand structures were selected based on the lowest binding affinity scores and the highest dipole moments. Table 5.5 summarizes the interactions between these phytochemicals and the target proteins, highlighting conventional, pi-donor, and CH-bond interactions. Active site amino acid residues such as Asparagine (Asn), Proline (Pro), Threonine, Valine (Val), Lysine (Lys), and Phenylalanine (Phe) were involved in different docking poses, contributing to the additive energy of the complexes through bond lengths, angles, and other energy components. The lowest binding affinity score was used to determine the best poses for all considered phytoconstituents. Accordingly,

the pose of Isoquercitrin (binding affinity score: CA2 = -6.5 kcal/mol, AVPR2 = -9.2 kcal/mol) with 3 H-bonds and 1 CH-bond was selected as the best pose. Similarly, the poses of Moracetin (CA2 = -5.3 kcal/mol, AVPR2 = -13.8 kcal/mol) with 3 H-bonds and 1 CH-bond, and Morin (CA2 = -6 kcal/mol, AVPR2 = -9.9 kcal/mol) with 1 H-bond were chosen. Rutin (CA2 = -6.6 kcal/mol, AVPR2 = -12 kcal/mol) showed 1 H-bond interaction. Additionally, the structures of carbonic anhydrase II in complex with furosemide (PDB ID: 3HS4), carbonic anhydrase II (CA2, PDB ID: 7agn), and arginine vasopressin receptor 2 (AVPR2, PDB ID: 7dw9) are presented in Fig. 5.13. The donor-acceptor interactions from the docking studies, demonstrating the best docking scores, revealed that Isoquercitrin, Moracetin, Morin, and Rutin formed strong interactions with both CA2 and AVPR2 in complex with furosemide.

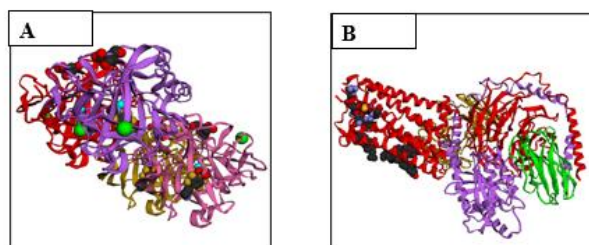
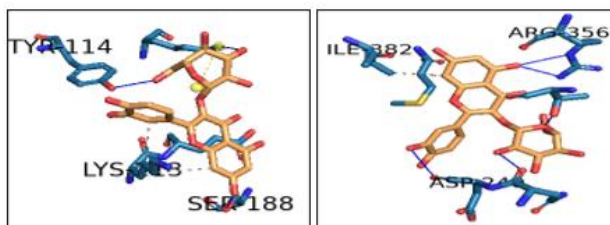


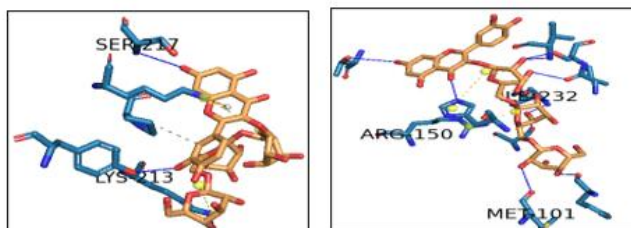
Fig No: 5.13 PDB Structure of CA2 (A) PDB Structure of AVPR2 (B)



CA2

AVPR2

Fig No: 5.14-Docking image of Isoquercitrin with receptors CA2 and AVPR2



CA2

AVPR2

Fig No: 5.15-Docking image of Moracetin with receptors CA2 and AVPR2

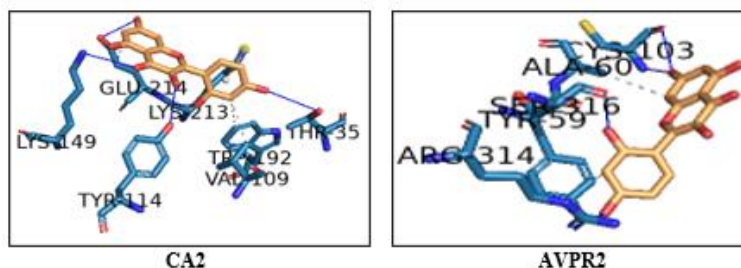


Fig No: 5.16-Docking image of Morin with receptors CA2 and AVPR2

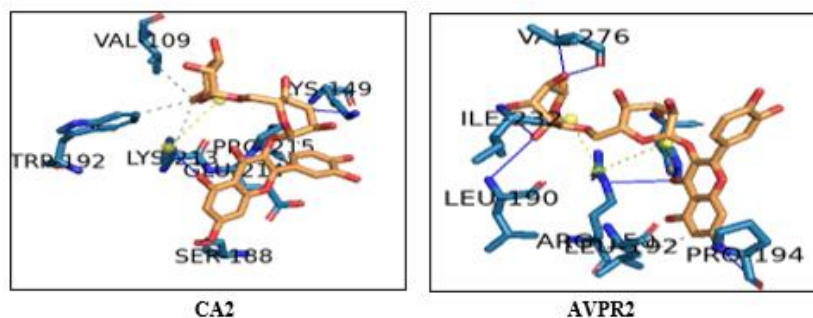


Fig No: 5.17-Docking image of Rutin with receptors CA2 and AVPR2

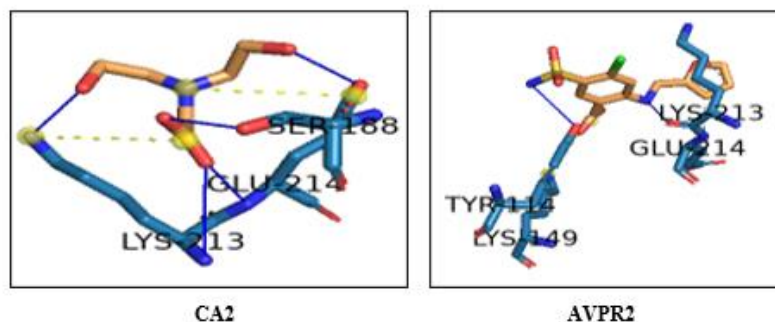


Fig No: 5.18-Docking image of Furosemide (standard) with receptors CA2 and AVPR2

Table No: 5.5 Hydrogen bond interaction and Binding affinity with receptors

Compound	Receptor	Binding affinity (kcal/mol)	Number of hydrogen bond Interactions
Isoquercitrin	CA2	-6.5	3
	AVPR2	-9.2	1
Moracetin	CA2	-5.3	3
	AVPR2	-13.8	1
Morin	CA2	-6	1
	AVPR2	-9.9	1
Rutin	CA2	-6.6	1
	AVPR2	-12	1
Furosemide (Std)	CA2	-4.8	1
	AVPR2	-4.8	2

5.4 IN-VITRO DIURETIC ACTIVITY

5.4.1 Carbonic anhydrase inhibition assay

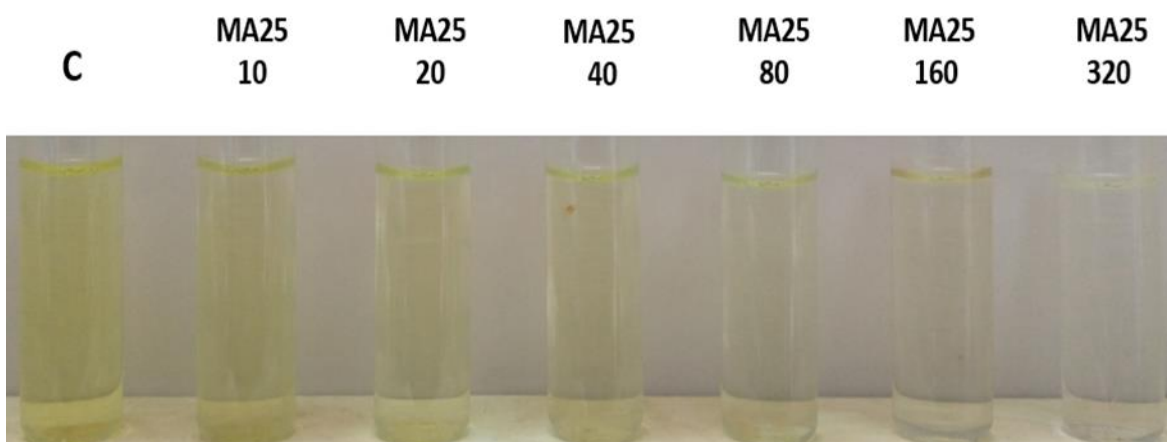


Fig No: 5.19 Different concentrations of standard (Acetazolamide)

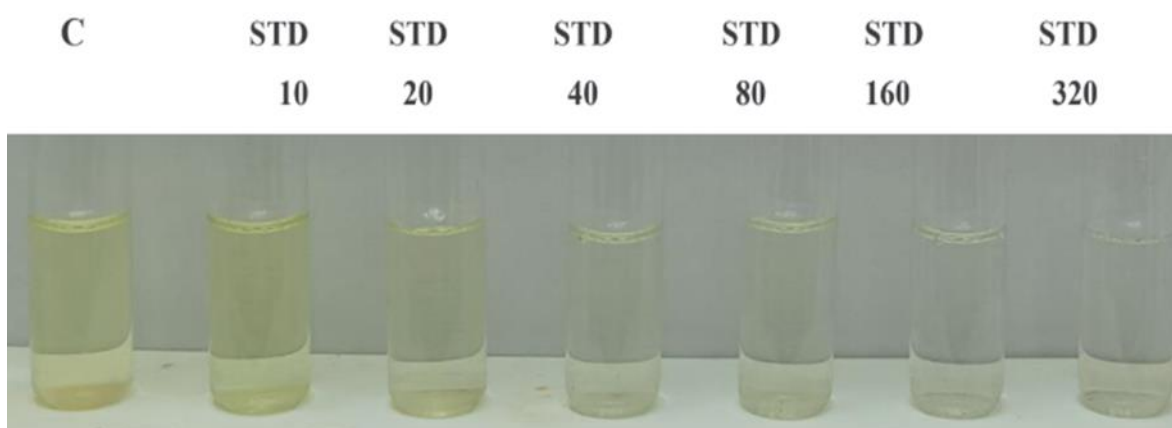


Fig No: 5.20 Different concentrations of *Morus alba* leaf extract

Table No: 5.6 Percentage inhibition curve of standard and plant extract

Sl. No	Concentration (µg)	MA extract	Standard (Acetazolamide)
1	10	5.071813285	29.91371045
2	20	16.11310592	48.94534995
3	40	34.64991023	65.14860978
4	80	50.85278276	75.98274209
5	160	65.57450628	83.79674017
6	320	80.78994614	88.54266539

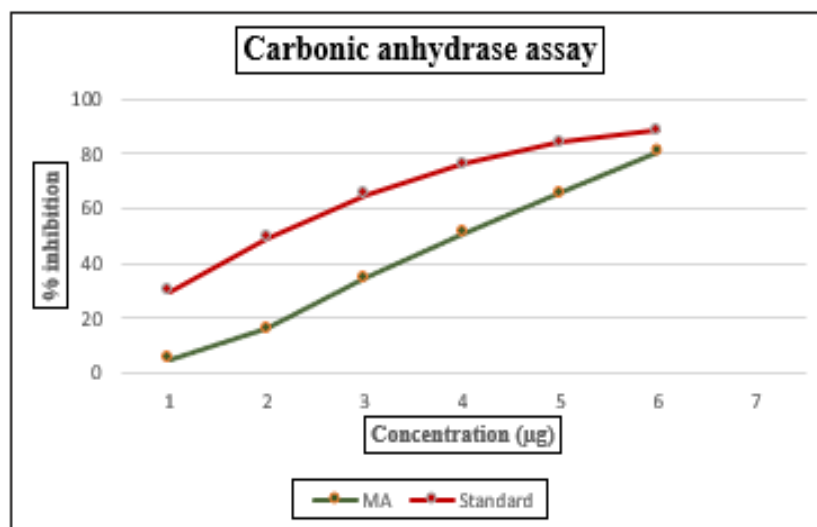


Fig No: 5.21 % inhibition of EEMA extract and Acetazolamide

The carbonic anhydrase inhibition activity of the EEMA extract and standard (acetazolamide) was found to be 80.22 µg/ml and 22.68 µg/ml respectively.

5.5 IN-VIVO DIURETIC ACTIVITY

5.5.1 Diuretic activity in rat [lipschitz model]

5.5.1.1 Effect of Urine pH

The control group, which received normal saline, exhibited a mean urinary pH of 7.183 ± 0.06 , whereas the standard group treated with furosemide showed a significantly higher mean pH of 7.767 ± 0.042 , indicating a pronounced alkalinizing effect ($p < 0.01$). The test group administered EEMA at 200 mg/kg demonstrated a mean pH of 7.417 ± 0.04 (* $p < 0.001$ vs control), while EEMA at 400 mg/kg resulted in a mean pH of 7.550 ± 0.03 (* $p < 0.05$ vs control). Overall,

both doses of EEMA led to an increase in urinary pH relative to the control group, showing a dose-dependent effect, although the increase was less marked than that observed with furosemide. The pH of freshly collected urine samples between the control and treated groups did not show a statistically significant difference.

Table No: 5.7 Effect of MA on pH of urine

Group	Drug to be administer	pH
Group I- Control group	Normal Saline 10mL/kg orally	7.183 ± 0.06
Group II- Standard group	Furosemide 10mL/kg orally	$7.767 \pm 0.042^{**}$
Group III-Test group I	EEMA 200mg/kg	$7.417 \pm 0.04^{***}$
Group V-Test group II	EEMA 400mg/kg	$7.550 \pm 0.03^*$

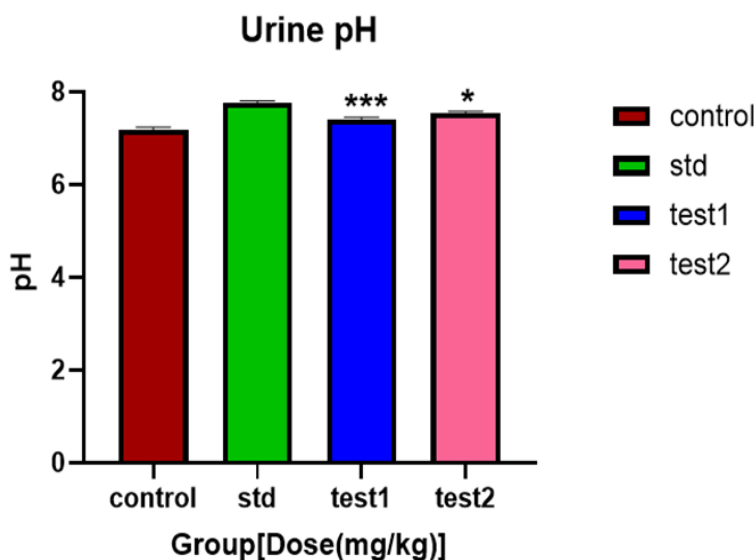


Fig No: 5.22 The effect of MA on urine pH

The values are expressed as mean $\text{Sem} \pm$ where $n=6$. The statistical analysis was carried out by Graph pad prism latest version 10.3.1. The comparison between groups was done by Student t test and multiple comparison were done by One-way Anova, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

5.5.1.2 Effect of Urine Volume

Urine output increased significantly in all treated groups compared to the control. The control group receiving normal saline had a mean urine volume of 1.55 ± 0.056 mL, whereas the standard group treated with furosemide showed a highly significant increase to 4.650 ± 0.009 mL (**** $p < 0.0001$). The test group administered EEMA at 200 mg/kg produced 2.56 ± 0.055 mL of urine

(**** $p < 0.0001$ vs control), while EEMA at 400 mg/kg resulted in 3.533 ± 0.071 mL (**** $p < 0.0001$ vs control). These results indicate that EEMA exhibits significant diuretic activity in a dose-dependent manner, with the higher dose approaching the efficacy of the standard drug.

Table No: 5.8 Effect of MA on urine volume

Groups	Drug to be administer	Urine Volume
Group I- Control group	Normal Saline 10mL/kg orally	1.55 ± 0.056
Group II- Standard group	Furosemide 10mL/kg orally	4.650 ± 0.009 ****
Group III-Test group I	EEMA 200mg/kg	2.56 ± 0.055 ****
Group V-Test group II	EEMA 400mg/kg	3.533 ± 0.071 ****

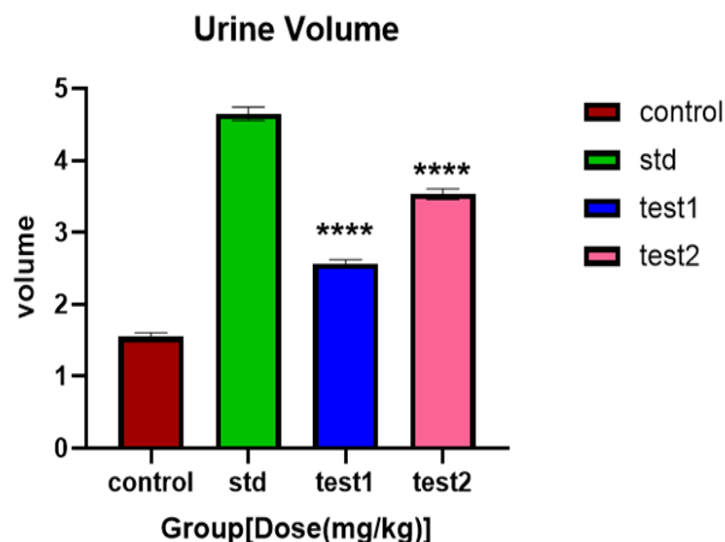


Fig No: 5.23 The effect of MA on urine volume

The values are expressed as mean $\text{Sem} \pm$ where $n=6$. The statistical analysis was carried out by Graph pad prism latest version 10.3.1. The comparison between groups was done by Student t test and multiple comparison were done by One-way Anova, * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$

5.5.1.3 Effect of urinary sodium, potassium, chloride levels in urine

The control group exhibited low urinary excretion of sodium, potassium, and chloride. Administration of EEMA at 200 mg/kg (Test group I) caused a slight increase in the excretion of all three electrolytes compared to the control, suggesting a mild diuretic effect. In contrast, EEMA at 400 mg/kg (Test group II) produced a pronounced increase in sodium and potassium excretion, along with a moderate rise in chloride levels, demonstrating a stronger diuretic effect that approached the activity of the standard drug furosemide.

Table No: 5.9 Effect of MA on urinary sodium, potassium, chloride levels in urine

Groups	Drug to be administer	Urine sodium [ppm]	Urine potassium [ppm]	Urine chloride [ppm]
Group I- Control group	Normal Saline 10mL/kg orally	35.00 $\pm$ 1.000	60.83 $\pm$ 2.880	61.33 $\pm$ 4.99
Group II- Standard group	Furosemide 10mL/kg orally	173.5 $\pm$ 2.861	118.7 $\pm$ 3.774	241 $\pm$ 10.41
Group III-Test group I	LEMA 200mg/kg	49.67 $\pm$ 2.060****	80.50 $\pm$ 1.998****	74.17 $\pm$ 1.99****

Group V-Test group II	LEMA 400mg/kg	130.00±1.138****	96.50±1.258****	88.17±2.22****
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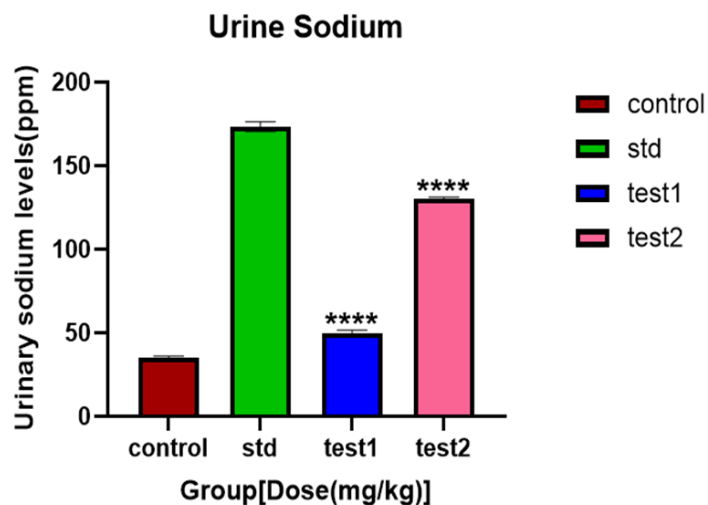


Fig No: 5.24 The effect of MA on urinary sodium level in urine

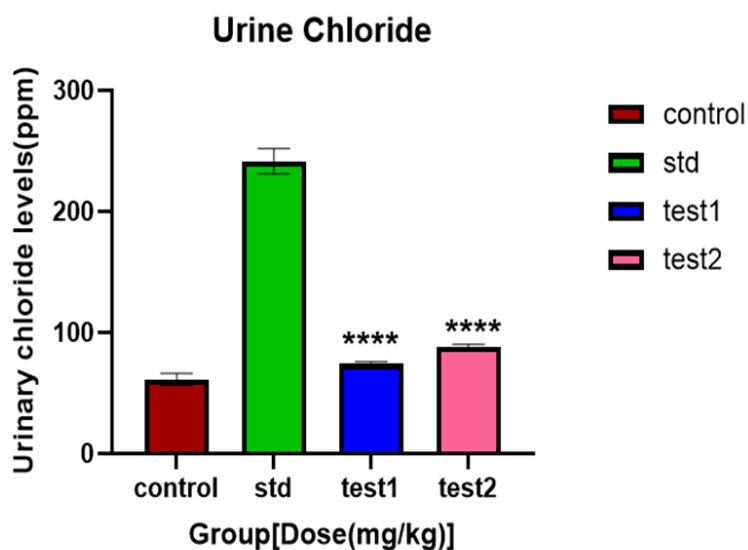


Fig No: 5.25 The effect of MA on urinary potassium level in urine

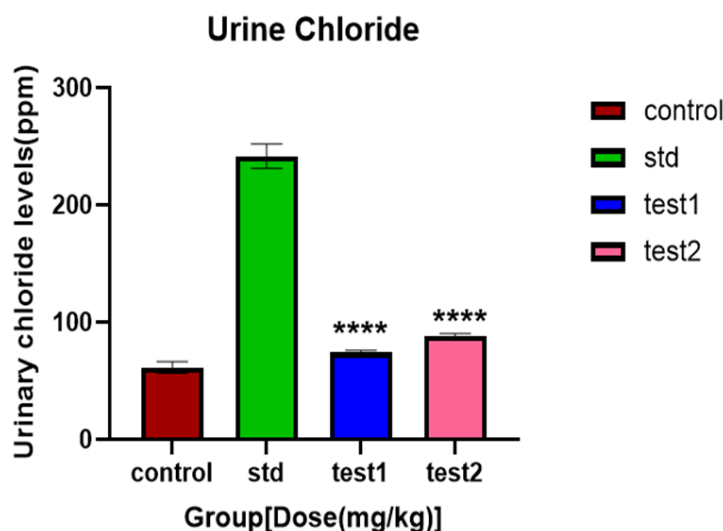


Fig No: 5.26 The effect of MA on urinary chloride level in urine

The values are expressed as mean $\text{Sem} \pm$ where $n=6$. The statistical analysis was carried out by Graph pad prism latest version 10.3.1. The comparison between groups was done by Student t test and multiple comparison were done by One-way Anova, * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$

5.5.1.4 Estimation of Diuretic index, Lipschitz value, Saluretic index, Na^+/K^+ ratio

The standard drug group demonstrated the highest diuretic and saluretic activity, confirming its

strong natriuretic effect. Both test groups showed significant improvements in diuretic activity compared to the control, reflecting the efficacy of the plant extract. The higher dose of the extract produced greater increases in urine output and electrolyte excretion than the lower dose, indicating a clear dose-dependent response. Moreover, the higher dose exhibited a better sodium-to-potassium excretion ratio, suggesting enhanced natriuretic efficiency. Overall, although the extract's activity was lower than that of the standard drug, it still displayed considerable diuretic potential, particularly at the higher dose.

Table No: 5.10 Effect of MA on Diuretic index, Lipschitz value, Saluretic index, Na^+/K^+

Groups	Drug to be administer	Diuretic index	Lipschitz value	Saluretic index		Na^+/K^+
				Na	K	
Group I- Control group	Normal Saline 10mL/kg orally	—	—	—	—	0.575
Group II-Standard group	Furosemide 10mL/kg orally	3.0	—	4.94	1.95	1.461
Group III-Test group I	LEMA 200mg/kg	1.6	0.55	1.41	1.32	0.617
Group V-Test group II	LEMA 400mg/kg	2.2	0.75	3.71	1.58	1.347

5.6 HISTOPATHOLOGY

Histopathology, a specialized branch of pathology, involves the microscopic study of tissue specimens to observe disease-related changes. It primarily emphasizes detecting structural and cellular alterations that aid in diagnosing disorders, monitoring disease progression, and assessing therapeutic outcomes. This technique is widely applied in both medical practice and research to identify infections, malignancies, tissue damage, and other pathological conditions.

Examination of the liver and kidney through histopathology provides important details about tissue organization and possible abnormalities. Under normal conditions, the liver displays clearly

defined hepatic lobules with central veins, radiating hepatocyte strands, and intact portal triads, while any evidence of degeneration, necrosis, inflammation, or fibrosis points to hepatic injury. Similarly, the kidney shows well-formed glomeruli, distinct Bowman's capsules, and organized renal tubules in healthy histology, whereas pathological features such as tubular casts, glomerular shrinkage, interstitial inflammation, or necrosis suggest renal damage. These microscopic investigations are critical in detecting organ injury and evaluating the impact of disease or treatment. In the present analysis, multiple sections of liver and kidney tissues were examined and revealed normal histological features, with all observations recorded at 100x magnification.

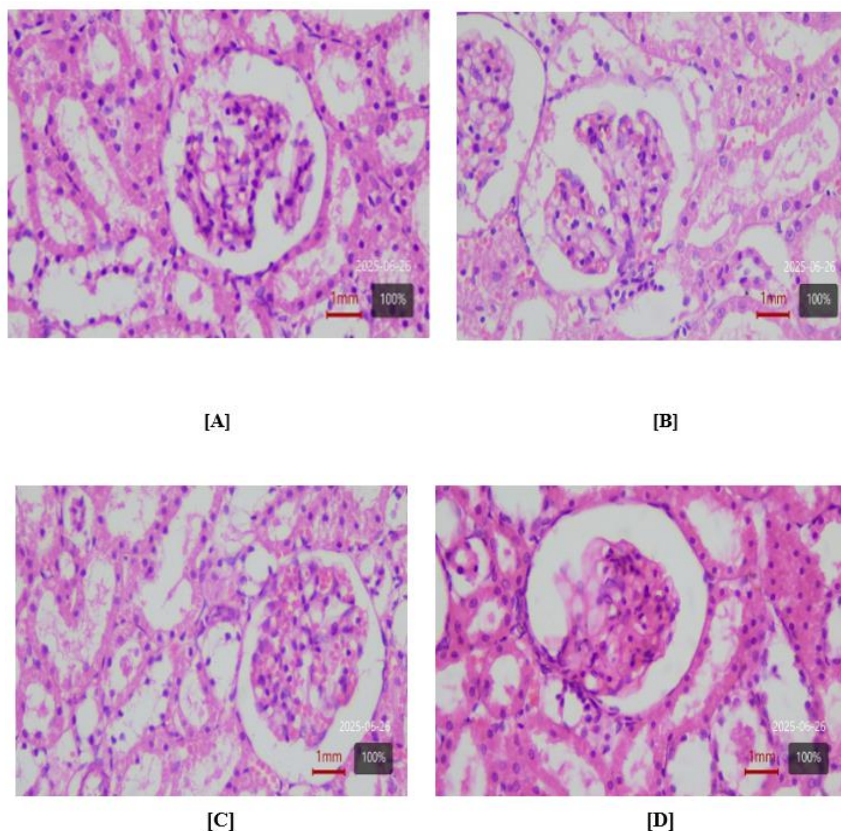


Fig No: 5.27 Photomicrographs of kidney [A] section of kidney showing normal histology for control group [B] section of kidney showing normal histology for standard group [C] section of kidney showing normal histology for EEMA 200 [D] section of kidney showing normal histology for EEMA.

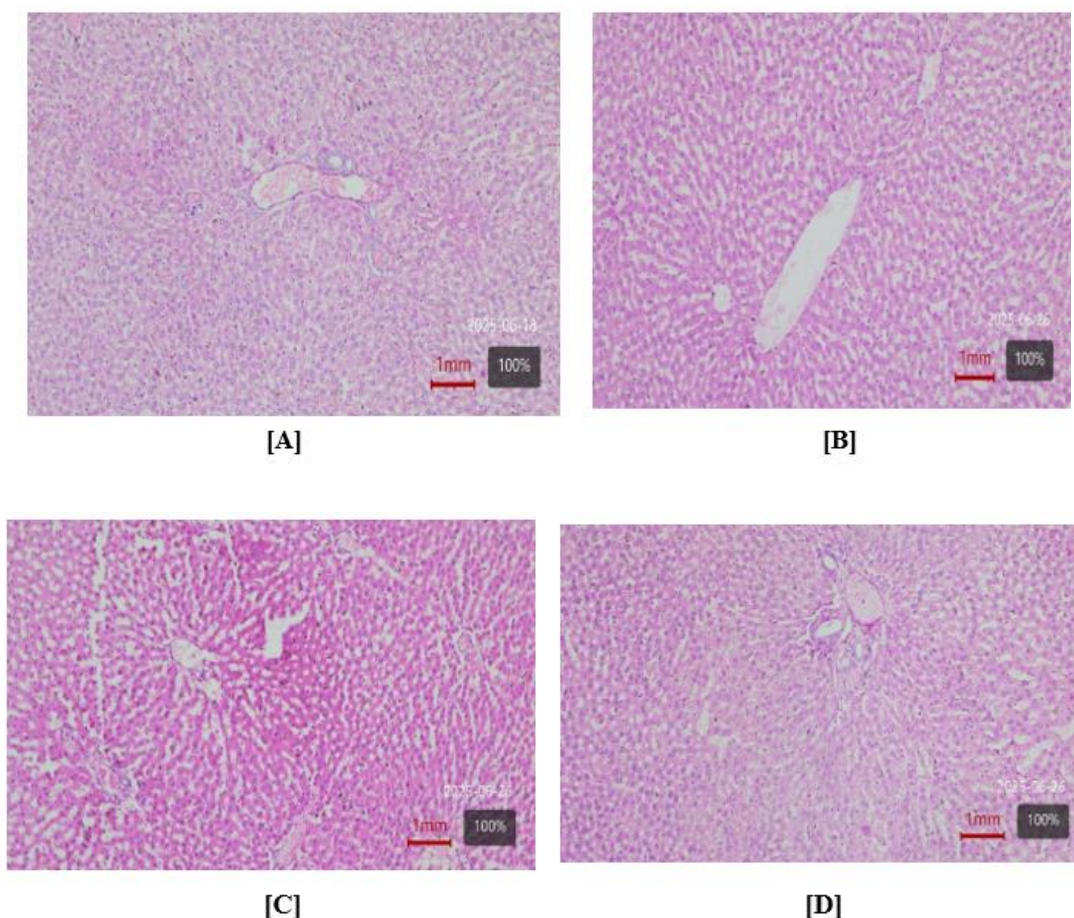


Fig No: 5.28 Photomicrographs of liver [A] section of liver showing normal histology for control group [B] section of liver showing normal histology for standard group [C] section of liver showing normal histology for EEMA 200 [D] section of liver showing normal histology for EEMA.

DISCUSSION

The findings of the present investigation provide compelling evidence that the ethanolic leaf extract of *Morus alba* (EEMA) exhibits notable diuretic activity, reinforcing its longstanding role in traditional medicine. The results obtained from phytochemical screening, docking analysis, in vitro enzyme assays, and in vivo animal studies collectively highlight the pharmacological potential of this plant as a natural diuretic agent. The detection of flavonoids, phenols, and tannins in the ethanolic extract is particularly significant, as these secondary metabolites have been widely reported to contribute to renal and cardiovascular regulation. This outcome is in agreement with

Namrata Verma et al. (2024), who described the phytochemical richness of *Morus alba* leaves, emphasizing flavonoids and phenolic acids as major contributors to its biological activities. The relatively high flavonoid content identified in the present study (42.82 mg/g in terms of quercetin equivalents) provides a biochemical foundation for its diuretic effects, since flavonoids are known to enhance renal blood flow, inhibit tubular sodium reabsorption, and modulate oxidative pathways that influence kidney function.

The docking studies performed in this research offer mechanistic insight into the molecular basis of *Morus alba*'s activity. Isoquercitrin, rutin, morin, and moracetin were shown to possess

strong binding affinities with carbonic anhydrase II (CA2) and vasopressin receptor 2 (AVPR2). These two targets are central to diuretic pharmacology: CA2 is crucial in bicarbonate reabsorption and acid–base balance, while AVPR2 mediates water reabsorption in the collecting ducts through the action of vasopressin. The strong interactions observed in docking simulations suggest that the extract exerts its effect through dual mechanisms—carbonic anhydrase inhibition and interference with vasopressin signalling. This mechanistic duality could explain the observed increase in urine volume, alkalinization of urine, and enhanced electrolyte excretion. Such findings align with the work of Wang et al. (2021), who emphasized that mulberry flavonoids influence multiple cellular pathways, including NF- κ B suppression and PI3K/Akt modulation, pointing to broad pharmacological relevance. Furthermore, Rahman et al. (2024) identified isoquercitrin as a major flavonoid with strong antioxidant and anti-apoptotic properties, supporting the likelihood that this compound contributes significantly to renal protection and diuretic activity in *Morus alba*.

The *in vitro* results from the carbonic anhydrase inhibition assay provide additional support for this mechanism. The ethanolic extract exhibited substantial enzyme inhibition, with an IC₅₀ of 80.22 μ g/mL. Although less potent than acetazolamide, the standard drug, this finding is noteworthy because plant extracts typically contain a mixture of compounds, and the effect observed represents the synergistic activity of multiple constituents. Previous reports by Hassan et al. (2020) described similar carbonic anhydrase inhibitory activity in herbal diuretics such as *Equisetum arvense* and *Hibiscus sabdariffa*. These parallels suggest that plant-based diuretics, including *Morus alba*, act at least partly through enzyme inhibition, providing scientific grounding for their traditional use.

The *in vivo* experiments in Wistar albino rats further reinforced the diuretic efficacy of the extract. Both doses of EEMA (200 mg/kg and 400 mg/kg) produced significant increases in urine volume, urinary sodium and chloride excretion, and urinary pH compared to the control group. Importantly, the effects were dose-dependent, with the higher dose approaching the efficacy of furosemide, the standard loop diuretic. This outcome is consistent with Khan et al. (2022), who reported that flavonoid-rich herbs such as those containing morin and kaempferol exert measurable diuretic effects in animal models. The dose-dependent increase in urine volume observed here suggests that *Morus alba* could be clinically useful in mild to moderate cases of fluid overload. While furosemide remains more potent, its side effects—electrolyte disturbances, ototoxicity, and nephrotoxicity—are significant drawbacks. In contrast, the safety of *Morus alba* extract, confirmed through histopathological evaluation of liver and kidney tissues, suggests it may be a safer alternative for long-term use.

Histopathological studies showed that both hepatic and renal tissues of treated animals maintained normal architecture, with no evidence of tubular necrosis, fatty degeneration, or inflammatory infiltrates. These findings strongly suggest that the ethanolic extract is non-toxic at the tested doses, even with repeated administration. Similar observations were reported by Ahmed et al. (2022) and Patel et al. (2021), who concluded that *Morus alba* extracts are generally safe and exert hepatoprotective and nephroprotective effects. This lack of toxicity is an important advantage over synthetic diuretics, which often require regular monitoring of renal function and electrolytes during prolonged therapy. The safety profile of *Morus alba* may therefore make it a suitable candidate for chronic conditions such as hypertension, congestive heart failure, or chronic

kidney disease, where diuretics are often prescribed for months or years.

The results also resonate with ethnomedicinal practices. Traditional systems such as Ayurveda, Siddha, and Chinese medicine have long employed *Morus alba* for treating oedema, urinary tract disorders, and hypertension. Singh et al. (2017) and Thakur et al. (2019) documented its use in alleviating swelling, urinary difficulty, and metabolic imbalances. The pharmacological findings of the present study provide a mechanistic basis for these traditional claims, thereby bridging ancient knowledge with modern science. The antioxidant and anti-inflammatory properties of flavonoids present in *Morus alba* may also complement its diuretic action by protecting renal tissues from oxidative stress, a factor implicated in diuretic resistance. This idea finds support in the work of Kumar et al. (2022) and Zhao et al. (2020), who highlighted the antioxidant and anti-inflammatory effects of *Morus alba* phytochemicals, underscoring their role in preserving kidney function.

The sustainability of *Morus alba* further enhances its therapeutic relevance. Being a plant cultivated widely for sericulture, its leaves are abundant, inexpensive, and environmentally sustainable. This contrasts sharply with synthetic drugs, which are costly and may not be readily available in rural or resource-poor settings. The development of standardized *Morus alba* extracts could therefore provide a low-cost, natural diuretic option for populations lacking access to conventional medicines. Additionally, its multi-functional pharmacology—combining diuretic, antioxidant, antidiabetic, and antihypertensive effects—makes it a versatile therapeutic candidate. Literature reports by Lee et al. (2023) and Singh et al. (2023) emphasize its potential in managing metabolic syndrome, diabetes, and hypertension, conditions

that often coexist with renal dysfunction and fluid overload. Thus, *Morus alba* could serve not only as a diuretic but also as a holistic agent addressing multiple disease pathways.

At the same time, the findings also highlight the need for further research. The diuretic effect of *Morus alba* was slightly weaker than that of furosemide, suggesting that while it may be effective for chronic management, it may not be suitable for acute conditions requiring rapid fluid removal, such as acute pulmonary oedema. Additionally, although docking and phytochemical analyses point to isoquercitrin and related flavonoids as the primary active constituents, the contribution of other compounds cannot be excluded. Fractionation and isolation of individual phytoconstituents, followed by pharmacological testing, would help clarify the specific bioactive molecules responsible for diuresis. Furthermore, pharmacokinetic studies are necessary to determine the bioavailability, metabolism, and excretion patterns of these compounds. These steps would facilitate standardization and dosage optimization, ensuring consistent therapeutic outcomes.

Clinical trials are also essential for translating these findings to human applications. While preclinical studies in animal models provide valuable insights, human physiology may respond differently due to variations in metabolism and hormonal regulation. Previous studies such as those by Singh et al. (2023) and Ahmed et al. (2022) have already demonstrated the hypoglycemic and antihypertensive potential of *Morus alba* in clinical settings, providing a foundation for extending investigations to its diuretic effects. Standardized extracts should be evaluated in randomized controlled trials to assess their safety, efficacy, and comparative effectiveness against conventional diuretics. Such



studies would not only validate its therapeutic potential but also help integrate it into modern medical practice as a complementary or alternative option.

Another interesting aspect of the present study is the correlation between the observed increase in urinary pH and the docking results with carbonic anhydrase. The alkalization of urine suggests inhibition of bicarbonate reabsorption, consistent with the action of carbonic anhydrase inhibitors. This mode of action is distinct from loop diuretics like furosemide, which act on the $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter in the loop of Henle. The combination of mild carbonic anhydrase inhibition and possible modulation of vasopressin receptor signaling suggests that *Morus alba* may have a unique pharmacological profile, combining features of different diuretic classes. This could potentially reduce the risk of diuretic resistance, a common problem in long-term therapy with single-mechanism drugs.

Taken together, the present findings underscore the therapeutic promise of *Morus alba* as a safe, effective, and sustainable diuretic agent. The integration of phytochemical analysis, molecular docking, in vitro enzyme assays, and in vivo pharmacological testing provides a comprehensive evaluation, confirming both efficacy and safety. The results are in line with a large body of literature highlighting the multifaceted pharmacological activities of *Morus alba*, ranging from antidiabetic and antioxidant to nephroprotective and cardioprotective effects. The dose-dependent increase in urine output, coupled with favourable histopathological outcomes, highlights its potential as a natural alternative to synthetic diuretics. Future work focusing on standardization, isolation of active compounds, and clinical validation will be essential to fully realize its potential.

SUMMARY

The study investigated the diuretic activity of *Morus alba* (white mulberry) leaves, emphasizing their potential as a natural alternative to synthetic diuretics. It begins with an overview of diuretics, their types, mechanisms, clinical uses, and side effects, followed by the significance of exploring plant-based remedies. The study highlights the phytochemical richness of *Morus alba* leaves, particularly flavonoids and phenolic compounds, which contribute to its pharmacological effects. Using Soxhlet extraction, phytochemical screening, molecular docking, and both in vitro (carbonic anhydrase inhibition assay) and in vivo (Lipschitz model in albino rats) methods, the ethanolic extract demonstrated significant diuretic activity, increasing urine volume, pH, and electrolyte excretion in a dose-dependent manner, comparable though less potent than the standard drug furosemide. Docking studies confirmed strong binding affinities of key compounds like isoquercitrin and rutin with diuretic targets. The findings suggest that *Morus alba* possesses promising diuretic potential, validating its traditional use in renal and hypertensive disorders, and supporting further exploration for safe, cost-effective, plant-based diuretic agents.

CONCLUSION

The present study demonstrates that the ethanolic leaf extract of *Morus alba* exhibits notable diuretic activity. Phytochemical screening confirmed the presence of flavonoids, phenols, and tannins, compounds recognized for their renal and cardiovascular benefits. The relatively high flavonoid content, particularly isoquercitrin, suggests that these constituents play a significant role in regulating diuretic effects. Molecular docking studies supported this observation, showing favorable binding affinities with carbonic anhydrase II and vasopressin receptor 2, indicating



potential mechanisms involving inhibition of tubular sodium reabsorption and regulation of water balance. In vitro analysis using the carbonic anhydrase inhibition assay demonstrated considerable enzyme inhibition, which aligns with the in vivo findings. Experiments in rats revealed that both 200 mg/kg and 400 mg/kg doses of the extract significantly increased urine output, enhanced electrolyte excretion, and raised urinary pH relative to controls. The responses were dose-dependent, with the higher dose producing effects approaching those of furosemide, although slightly less potent. Histopathological examinations confirmed the absence of adverse changes in liver and kidney tissues, suggesting that the extract is safe at the tested doses. This safety profile enhances the therapeutic potential of *Morus alba*, particularly for prolonged use. While synthetic diuretics such as furosemide are more potent, they are often associated with side effects, including electrolyte disturbances and nephrotoxicity, whereas *Morus alba* extract provided effective diuretic action without detectable toxicity. These findings support the traditional use of *Morus alba* in managing edema, urinary tract disorders, and fluid retention. The extract offers a natural and cost-effective alternative, particularly valuable in resource-limited settings. Future research should focus on isolating the active compounds, optimizing dosages, and evaluating clinical efficacy in human trials. In conclusion, *Morus alba* demonstrates substantial potential as a safe, effective, and sustainable herbal diuretic.

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