EVALUATION OF ANTILITHIATIC ACTIVITY OF AQUEOUS EXTRACT OF LEAVES OF *Clitoria ternatea*

Dissertation

Submitted to

The Tamil Nadu Dr. M.G.R. Medical University, Chennai.

In Partial fulfillment for the award of the degree of

MASTER OF PHARMACY

in

PHARMACOLOGY

by

Reg No:26113391

DEPARTMENT OF PHARMACOLOGY

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OCTOBER - 2013

DECLARATION

I hereby declare that this thesis work entitled **" EVALUATION OF ANTILITHIATIC ACTIVITY OF AQUEOUS EXTRACT OF LEAVES OF *Clitoria ternatea* "** submitted to the Tamil Nadu Dr. M.G.R. Medical University, Chennai was carried out by me in the Department of Pharmacology, Ultra College of Pharmacy, Madurai under the valuable and efficient guidance of **Mr.N.SRIDHAR, M.Pharm**., Asst. Professor, Department of Pharmacology, Ultra College of Pharmacy, Madurai during the academic year Nov 2011- Oct 2013, I also declare that the matter embodied in it is a genuine work and the same has not found formed the basis for the award of any degree, diploma, associateship, fellowship of any other university or institution.

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1.

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DEDICATED TO MY

BELOVED FAMILY,

TEACHERS AND

FRIENDS

ACKNOWLEDGEMENT

Completing task is never a one man effort. It is often result of invaluable contribution of a number of individual in a direct or indirect manner. This suitably applied to my dissertation work.

I explain my extreme sense of gratitude, profound thanks to my guide **Mr.N.SRIDHAR, M.Pharm.,** Department of Pharmacology, Ultra College of Pharmacy, Madurai for his precious guidance, encouragement and abundant help, his enthusiastic and inspiring discussion and timely suggestions which proved for the success of this work.

I do feel, highly elated in manifesting a sense of gratitude to my honourable principal ***Prof*.K.R.Arumugam, M.Pharm.,** who permits me to do this project and showers his blessings and guidance whole party in every walk of my successful career.

It is my privilege and honour to extend my profound gratitude and express my indebtedness to our Dean **Dr.C.Vijaya, M.Pharm., Ph.D.,** Ultra College of Pharmacy, Madurai for the constant inspirations, valuable advice, help, encouragement and innovative ideas throughout the course of the project.

My heartiest acknowledge renderd to **Dr.A.Babu Thandapani, M.Pharm., Ph.D., Dr.K.G.Lalitha, M.Pharm., Ph.D., Mr.N.Chandran, M.Pharm., Mr.K.Natarajan, M.Pharm., Mr.T.Regupathi, M.Pharm., and**

Mr.V.Sivananad, M.Pharm., Ultra College of Pharmacy, Madurai for their valuable suggestions throughout my thesis work.

Ms. Manimegalai and Ms. Vidhya., Laboratory attenders **Mrs. Thangamani, Mr.S.Sonai, Ms.N.Poongodi** and **Mrs. Masila** in Ultra College of Pharmacy, Madurai.

I extend my sincere thanks to my classmates **Mr.Venkatraghavan** and **Mrs.Mahalakshmi** who helped me throughout this project..

I am very much grateful to my seniors **Ms.Neethu, Mr.kannan,**

I offer flowers of gratitude to my daughter and son who have been the source of strength throughout my life.

I had throughout my project, the solid and continued support of all my ***friends*** and ***colleagues.*** I wish them all my heartfelt best wishes in all their endeavours.

CONTENTS

INTRODUCTION

"No stretch of chemical or physical imagination will permit so heterogeneous a group of compounds (as renal stones) to be ascribed to a common origin, or their disposition in the kidney, ureter or bladder to be uniformly charged to an identical cause."

- (Howard Kelly)

India has been referred to as the medicinal garden of the world. India comes under the 12 mega biodiversity centers having 45,000 plants species. In India around 20.000 medicinal plants species have been recorded, but around 500 traditional communities use 800 plant species for curing the diseases. Today around 50% of world population is totally depends upon the plant derived products as a primary health care with no side effects (Ankur et al., 2010).

Nature bestowed our country with an enormous wealth of medicinal plants; ants have been used as traditional healthcare system from the centuries. The World health Organization (WHO) has listed 20,000 medicinal plants in globally in which contribution of India is 15-20 %. The WHO reported that 80% of global countries depend on the medicinal plants. A large body of evidence has collected to show potential of medicinal plants used in various traditional systems. In the last few years more than 13,000 plants have been studied for the various diseases and ailments in all over the world (Ankur et al., 2010).

Nephrocalcinosis and uro (nephro) lithiasis frequently coexist and the terms often loosely combined when describing patients with urinary stone disease, whether they are etiologically distinct is unclear, although it is generally believed nephrocalcinosis represents one end of the spectrum of urinary stone disease, ever, although nephrocalcinosis is often associated with urinary stones do not have macroscopic nephrocalcinosis (David.A.Warrell, 2003).

Stone formation in the kidney is one of the oldest and most wide spread disease known to man. Urinary calculi have been found in the tombs of Egyptian mummies dating back to 4000 BC and in the graves of North American Indians from 15000-1000 BC. Reference to stone formation is made in the early Sanskrit documents in India between 3000 and 2000 BC.

Urinary stone disease has afflicted humankind since antiquity and can persist, with serious medical consequences, throughout a patient's lifetime. In addition, the incidence of kidney stones has been increased in western societies in the last five decades, in association with economic development (Bahuguna et al., 2009).

The recurrence of urolithiasis represents a serious problem, as patients who have formed a stone are more likely to form another, and thus stone prevention is highly recommended. Currently, open renal surgery for nephrolithiasis is unusual and usual only rarely, since the introduction of ESWL (Extracorporeal Shock Wave Lithotripsy). which has revoltunised urological practice and almost become the standard procedure for eliminating kidney stones. However, in addition to the traumatic effects of shock waves, persistent residual stone fragments, and the possibility of infection, suggest that ESWL(Extracorporeal Shock Wave Lithotripsy) may cause acute renal injury, a decrease in renal function and an increase in stone recurrence. Further more, although some drugs used to prevent the disease have some positive effects, they are not effective in all and often have adverse effects that compromise their use in long-term Medicinal treatment. Alternative treatment using phytotherapy has been sought; indeed, in recent years there has been a resurgence of interest in medicinal plants that are effective safe and culturally acceptable (Atmani, et al., 2003).

Many remedies have been employed during ages to treat renal stones. Most of remedies were taken from plants and proved to be useful, though the

rational behind their use is not well established except for a few plants and some proprietary composite herbal drugs and they are reported to be effective with no side effects. The present day medical management of nephrolithiasis is either costly or not without side effects. Hence the search for antilithiatic drugs from natural sources has greater assumed importance (Atef M. Al-Attar., 2010).

The present study evaluated for the possible therapeutic potential of aqueous extract from the leaves of *Clitoria ternatea* (L) in experimentally induced calcium oxalate urolithic rats and its diuretic potential.

The urinary system is the main excretory system and consists of the following structures. 2 kidneys, 2 ureters , Urinary bladder, Urethra. The urinary system plays a vital part in maintaining homeostasis of water and electrolyte concentrations with the body. The kidneys produce urine that contains metabolic waste products, including the nitrogenous compounds urea and uric acid, excess ions and some drugs.

1. KIDNEY

The kidneys lie on the posterior abdominal wall, one on each side of the vertebral column, behind the peritoneum and below the diaphragm. They extend from the level of the 12^{th} thoracic vertebra to the 3^{rd} lumbar vertebra, receiving some protection from the lower rib cage. The right kidney is usually slightly lower than the left probably because of the considerable space occupied by the liver.

Kidneys are bean-shaped organs, about 11 cm long, 6 cm wide, 3 cm thick and 150g. They are embedded in, and held in position by, a mass of fat. A sheath of fibroblastic renal fascia encloses the kidney and the renal fat (Ross and Wilson. 2006).

Functions

- Formation and secretion of urine.
- Production and secretion of erythropoietin, the hormone that controls formation of red blood cells.
- Production and secretion of rennin, an important enzyme in the control of blood pressure.

2.RENAL DISEASE

Renal disease can be classified into five different physiological categories:

- Acute renal failure, in which the kidneys stop working entirely or almost entirely.
- Chronic renal failure, in which progressively more nephrons are destroyed until the kidneys simply cannot perform all the necessary functions.
- Hypertensive kidney disease, in which vascular or glomerular lesions cause hypertension but not renal failure.
- Nephrotic syndrome, in which the glomeruli have become far more permeable so that large amounts of protein are lost into the urine,
- Specific tubular abnormalities that cause abnormal reabsorption or lack of reabsorption of certain substances by the tubules.

2.1. Acute Renal Failure

"A clinical disorder of sudden cessation of renal function characterised by uraemia. and disturbance in body fluid and electrolyte balance, with or without 'accompaniment of oliguria."

The causes of acute renal failure can be divided into three main categories
a) Acute renal failure resulting from decreased blood supply to the kidneys; this condition is often referred to as pre renal acute renal failure to reflect the fact that the abnormality occurs in a system before the kidneys. This can be a consequence of heart failure with reduced cardiac output and low blood

pressure or conditions associated with diminished blood volume and low blood pressure, such as severe haemorrhage.

b) Intrarenal acute renal failure resulting from abnormalities within the kidney itself. including those that affect the blood vessels, glomeruli, or tubules.

c) Postrenal acute renal failure**,** resulting from obstruction of the urinary collecting system anywhere from the calyces to the outflow from the bladder. The most common causes of obstruction of the urinary tract outside the kidney are kidney stones caused by precipitation of calcium, urate or cystine (Guyton & Hall, 1991).

2.2. Chronic renal Failure

"A symptom complex resulting from renal insufficiency (uraemia), and is characterized by nitrogen retention acidosis and anaemia". The condition develops from a number of renal and extra renal disorders , involving the renal parenchyma or obstruction of the excretory tract, which include chronic nephritic syndrome , chronic pyelonephritis, diabetic glomerulosclerosis, acute tubulo interstitial nephritis , benign nephrosclerosis polycystic kidney disease . bilateral cortical necrosis, massive renal infarct, addison's disease. disseminated lupus erythematous infections and exposure to nephrotoxins. It also develops from the conditions, which predispose to acute renal failure. On the other hand**,** there may not be any previous precipitating factor in the development of the condition (Hossain. 2004).

2.3. Urinary tract obstruction

Urinary tract obstruction or obstructive uropathy can occur at any point in the urinary tract, from the kidneys to the urethral meatus. Certain points along this path are more susceptible to obstruction. The three points of narrowing along the ureter include the uretero pelvic junction (UPJ), the

crossing of the ureter over the area of pelvic brim (the iliac vessels), and the uretero vesicle junction (UVJ). It can develop secondary to calculi, tumors, strictures, and anatomical abnormalities. (Obstructive uropathy can result in pain, urinary tract infection, loss in renal function, or possibly, sepsis or death.

Urinary tract obstruction impedes urine flow. This obstruction causes distention of the urinary tract proximal to the point of obstruction. The distention is caused by increased pressure and can result in pain, which may be the first sign of obstruction. Distortion of the urinary tract and renal failure can develop; the severity depends on the degree and duration of obstruction. When the urinary tract is obstructed, urine stasis can occur; predisposing to urine infection. The clinical presentation of urinary tract obstruction varies with the location, duration, and degree of obstruction. Thus, a thorough history and physical examination are key in the patient evaluation (Edward David Kim et al.,2005).

3.KIDNEY STONES

Calculi, or renal stones (nephrolithiasis), are masses of crystals and protein and are a common cause of urinary tract obstruction in adults. Renal stones account for 1 of 1000 hospitalizations, with an incidence at autopsy of approximately 5%. A significant number of patients are treated in outpatient settings. Approximately 1 % of the U.S. population will have a renal will have a renal stone at some time. Three major kinds of renal stones are

(1) Calcium oxalate
(2) Struvite (magnesium, ammonium, phosphate) and
(3) Uric acid

3.1. Epidemiology

Kidney stone disease is a multi factorial disorder resulting from the combined influence of epidemiological, biochemical and genetic risk factors. Kidney stones are of four types. The overall probability of forming stones differs in various parts of the world and is estimated as 1-5% in Asia, 5-9% in

Europe, 13% in North America and the recurrence rate of renal stones about 75% in 20 years span. It occurs both in men and women but the risk is generally high in men and is becoming more common in young women (Sandhya et al., 2010)

The incidence of urolithiasis is very common in Northern India compared to southern state. It is speculated that higher incidence may be due to wheat diets. People living in rocky areas, where the climate is hot and dry, seem to be more to urinary calculi disease (Chitme, et al., 2010)

The temperature rise worldwide due to the effects of global warming, it has been predicted that there could be an increase of 1.6-2.2 million lifetime cases of kidney stone by 2050, particularly in the southeast regions of the USA (Andrew P.Evan.,2010)

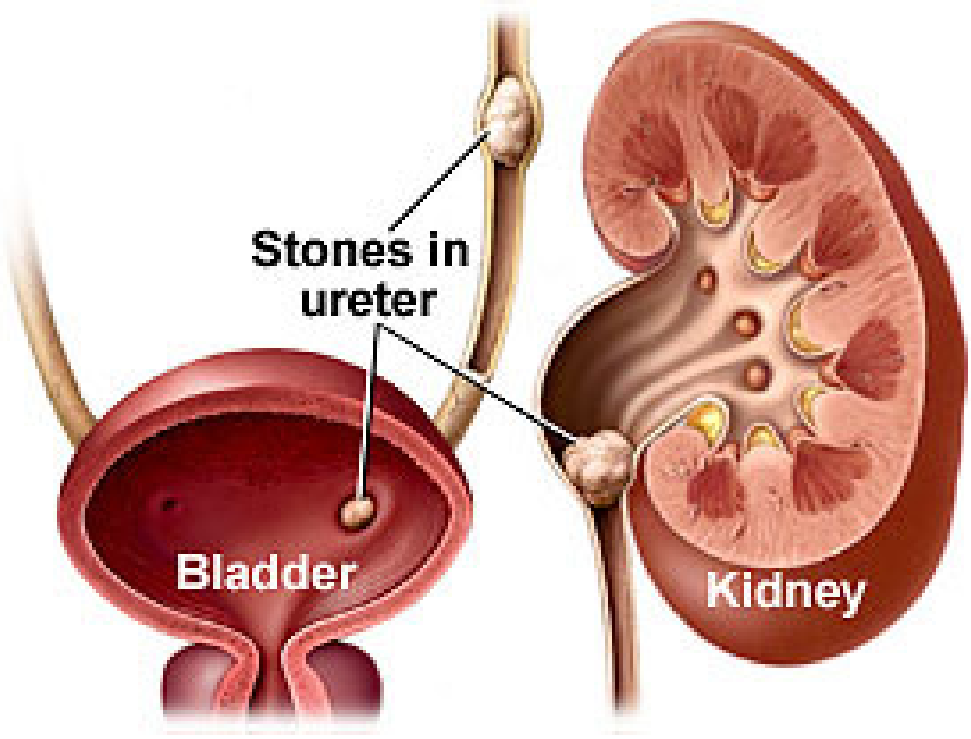


Figure:1Kidney Stone

Etiology

Several Etiological factor contribute to the pathogenesis of stone formulation

- **Geography**

Kidney stone incidence varies in different parts of the world, thus projecting the significance of the stone belt areas. The effect of geography on the incidence of stone formation may be direct, through its effect on temperature; high temperatures increase perspiration, which may result in concentrated urine, which in turn promotes increased urinary crystallization.

- **Age & Sex**

The disease affected all age groups from less than 1 year old to more than 70, a male to female ratio of 2:1.

- **Nutritional aspects**

An unbalanced diet or particular sensitivity to various foods in stone formers can lead to urinary alterations such as hypercalciuria, hyper oxaluria hypocitrauria, hyperuricosauria, and excessive acid urinary pH.

- **Diet**

Some reports have described that vegetarians are at lower risk for stone formation in contrast to non-vegetarians

- **Water intake**

Super saturation of the urinary environment with stone -forming constituents is a prerequisite for calculus formation and increased fluid consumption results in excretion of higher volume of urine, which is less supersaturated with stone –forming constituents.

- **Body weight**

Overweight condition and obesity was found in 59.2% of men and 43.9% of women and both these conditions were strongly associated with an elevated risk of stone formation in both genders due to increased urinary excretion of promoters but not inhibitors of calcium oxalate stone formation.

The overweight and obese men are more prone to stone formation than overweight women.

- **Kidney stone and other diseases**

It has been proposed that essential hypertension, cardiovascular diseases (CVD), diabetes, and other medical conditions predispose to stone disease

- **Recurrence**

The recurrent nature of stone disease is a well-recognized Clinical problem. Male gender, multiple stones, stone location, residual fragments and some anatomic or functional urinary tract abnormalities are known to be major risk factors for recurrence.

- **Occupation**

The role of occupation in stone formation is highly debated. Kidney-related complications are on the increase because of geographic factors residence in the "stone belt, occupation related lifestyle changes - in case of indoor occupation - sedentary habits, stress, unhealthy dietary plan in terms of healthy or over healthy food intake, irregular food habits and fluid intake (intake of juices and beverages instead of water) or the other spectrum of physical manual labour - involving working outside exposed to heat and sun, low socioeconomic status, malnutrition and reduced fluid intake." Some experts speculated that this increased risk might be due to a hormone called vasopressin, which is released during stress, which increases the concentration of urine.

Molecular Aspects

Stone disease is a multifactorial disease; the causes of calcium oxalate stones are hetrogeneous and might involve both genetic and environmental factors.

Although extensive genetic studies were carried out, no chromosomal mapping has been conducted in patients with stones and idiopathic hypercalciuria (IH). The only conclusive evidence through genetic studies is that urolithiasis is a polygenic defect and partly penetrative (Sandhya et al., 2010)

3.3. Mechanism of calcium oxalate renal stone formation

Pathophysiologic mechanisms of stones are complex, mainly because stone disease- polygenic, multifactorial disorder that involves an interrelationship between kidney, bone, and intestine. Much effort has been undertaken in recent years to delineate the pathophysiologic process that leads to the formation of renal calculi. There are distinct stone phenotypes and the cascade of events leading to kidney stone formation varies depending on this phenotype. Different mechanisms of stone formation have been described for numerous stone types and clinical situations. (Gnessin et al., 2010).

The formation of renal stones is a consequence of increased urinary supersarturation with subsequent formation of crystalline particles. Since most of the solid particles crystallizing within the urinary tract will be excreted freely, particle formation is by no means equivalent to symptomatic stone disease. However, when solid particles are retained within the kidney, they can grow to become full-size ones. Crystal-cell interaction is the next step, and is also promoted by renal tubular injury. Since crystal formation is a common phenomenon in human urine and crystaluria per sec is harmless, abnormal retention of formed particles must occur when kidney stones form. Thus, crystal-cell interactions may be highly relevant. The crystals that are internalized in the interstitium undergo growth and aggregation, and develop into renal stones. Each of these processes is described in detail below.
(Tsujihata et al., 2007)

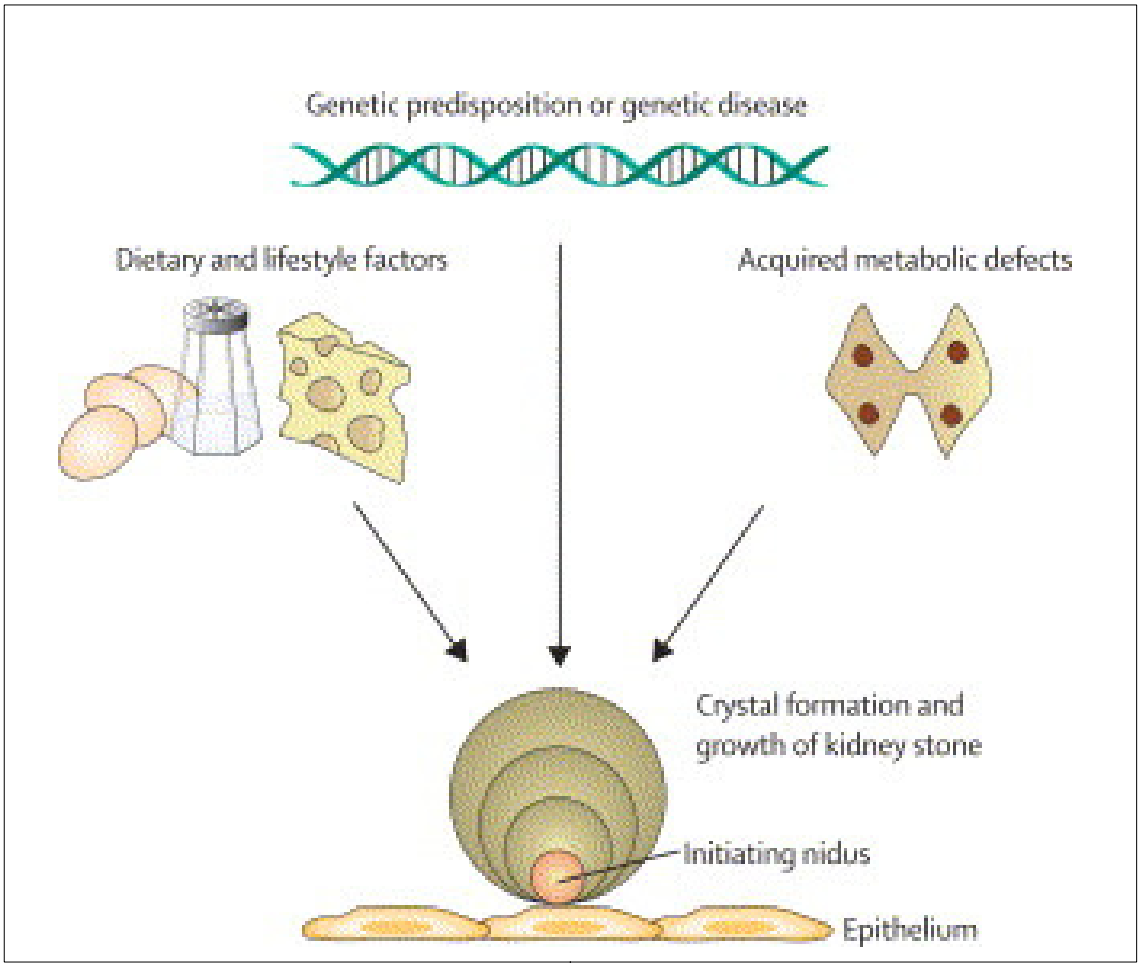


Figure 2: Pathogenesis of Kidney Stones

Using calcium oxalate stones as a model, three categories of factors (genetic, metabolic, and dietary) act in conjunction or in isolation to lead to kidney stone formation. The process probably needs an initiating nidus on the epithelium, which provides the platform for crystallization and growth. The defect probably includes lesions in the cells and luminal factors.

3.3.1.Urinary super saturation and crystallization

Urinary super saturation is the driving force behind crystal formation in the kidneys. Since formation of crystalline particles must obviously start from super saturation. It is undoubtedly essential for stone formation. Indeed, stone formers tend to excrete urine that is more supersaturated than that of non-stone formers. Humans excrete millions of urinary crystals daily, indicating at least transient development of super saturation. It has been suggested that with a transit time across the kidney of 5-10 min, residence time is too short for crystals to nucleate and grow large enough to be trapped. The inner diameter of the various segments of the renal tubules ranges from 15 to 60 mm. Calcium oxalate crystals, growing at the rate of 1-2 mm/min, cannot grow larger than a

few microns and are therefore excreted with urine without causing stone development. In tubular fluid and urine, crystallization processes are largely dependent on solution composition. A variety of urinary constituents may affect solution super saturation because of their activity as chelaters. For instance, by forming soluble complexes with calcium and oxalate respectively, citrate and magnesium reduce free ion activity and the relative super saturation of calcium oxalate.

3.3.2. Crystal Nucleation

The initial step in the transformation from a liquid to a solid phase in a supersaturated solution is called nucleation. This process begins with the coalescence of salts in solution into loose clusters that may increase in size by addition of new components or clusters. In vitro and in vivo studies have shown that renal tubular cell injury can promote crystallization of Calcium oxalate crystals by providing substances for their heterogeneous nucleation. In vitro cell degradation following renal tubular cell injury produces numerous membrane vesicles, which have been shown to be good nucleators of calcium crystals. In vivo crystals observed in the renal tubules of hyperoxaluric rats are always associated with cellular degradation products.

3.3.3. Crystal growth

Once crystal nucleus has achieved a critical size and relative super saturation remains above 1, overall free energy is decreased by adding new crystal components to the nucleus. This process is called crystal growth. Crystal growth is one of the prerequisites for particle formation and thus for stone formation. In each step of stone formation., crystal growth and aggregation have important function. The crystal surface binding substance, which is found in Calcium oxalate crystals generated from whole human urine, is a strong inhibitor of Calcium oxalate crystal growth and contains human serum albumin, 1-acid glycoprotein, α1-microglobulin, 2-HS glycoprotein, retinol binding protein. transferrin, Tamm-Horsfall glycoprotein, and

prothrombin. However, it has been suggested that the importance of crystal growth for Calcium oxalate, the most abundant stone component, is questionable. Since the rate of Calcium oxalate crystal growth is low and the transit time of tubular fluid through the kidney amounts to only several minutes; it has been calculated that the probability of a single particle achieving a pathophysiologically relevant size by the process of crystal growth alone is extremely low**,** even if growth proceeds at an uninhibited rate of 2 mm per minute. The inhibitory effect of fibronectin (FN), a multifunctional α2-glycoprotein distributed throughout the extracellular matrix and body fluids, on Calcium oxalate crystal growth is small, considering the quantity normally excreted. Fibronectin at a concentration of 0.5 mg/mL only 9.9% inhibition of Calcium oxalate crystal growth. (Tsujihata M et al., 2007)

3.3.4. Crystal aggregation

The crystals of solution is sticking together to form larger particles is called aggregation process. Some researchers have proposed that crystal aggregation is the most important step in stone formation. Although crystal growth is a definite step of Calcium oxalate renal stone formation; the process of growth is so slow that crystals cannot become large enough to obstruct the renal tubules and be retained there by this mechanism alone, as several minutes are required for the tubular fluid to pass through the kidney. For this reason, the more critical step is thought to be crystal aggregation. All models of Calcium oxalate urolithiasis concede that crystal aggregation is probably involved crystal retention within the kidneys, since aggregation of crystals can have a considerable effect on particle size, and aggregated crystals are commonly found in urine and renal stones.

Crystal aggregation is promoted by viscous binding, implying that crystal foreign compounds with multiple binding sites, such as abnormally self-aggregating Tamm- Horsfall glycoprotein or other macromolecules, attach to crystal surfaces and act as a kind of glue. The inhibitory effect of Fibronectin

on Calcium oxalate crystal aggregation was found to be 47.7% at the 0.5 mg/mL physiological concentration of excreted Fibronectin.

3.3.5. Crystal-cell interaction

The mechanisms of crystal-cell interaction are thought to be very complex, and many of them remain unexplored. Crystallization is caused by the condition of urinary super saturation. Then, the crystals that have formed attach to renal tubular epithelial cells and are taken into them. The process of attachment or endocytosis of crystals to renal tubular cells is generally known as crystal-cell interaction. Adhesion of 14C-labeled calcium oxalate monohydrate (COM) crystals was detected as early as 30's after their addition to cultures of BSC-1 cells, followed by their uptake, whereas calcium phosphate crystals did not exhibit uptake to the same extent. The structural characteristics of the binding and uptake of COM crystals by BSC-1 cells have been characterized by scanning electron microscopy (SEM). Microvilli on the apical cell surface appear to make initial contact with the crystal before its internalization. Transmission electron microscopy (TEM) confirmed that endocytosis of COM crystals by BSC-1 cells occurs as early as 30 min after exposure. These structural and functional studies of crystal-cell interactions in culture indicate that COM crystals rapidly adhere to microvilli on the cell surface and are subsequently internalized. The behavior of these cells in vitro provides a dynamic model to explain the presence of intracellular Calcium oxalate crystals in the kidneys of patients with hyperoxaluria.

In recent years, a number of investigators have emphasized that crystal-cell interactions, including crystal attachment and endocytosis, are important processes in Calcium oxalate renal stone formation. Crystal-cell interactions are now thought to be extremely important in physiological crystal retention and the early stages of Calcium oxalate renal stone formation. Thus, Calcium oxalate crystals may be retained in the kidney to form stones by binding to the

apical surface of tubular cells and subsequently undergoing endocytosis (Tsujihata et al., 2007).

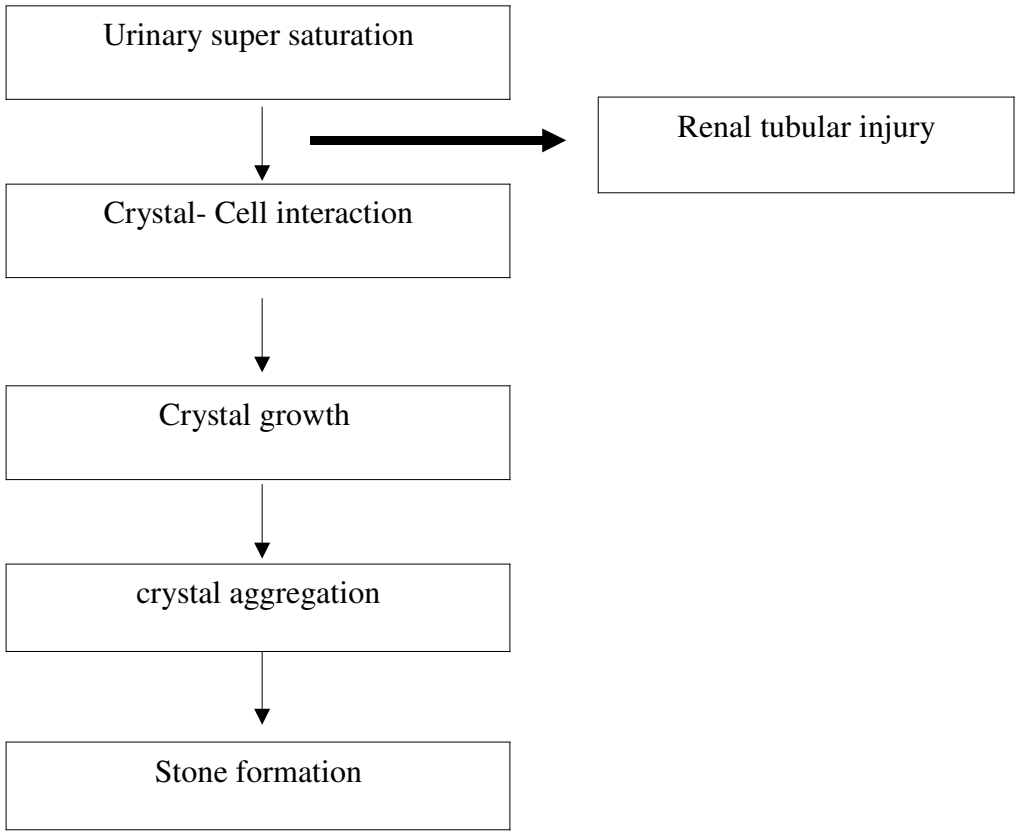


Fig 3: Scheme of the process of calcium oxalate renal stone formation

Types of stone

Four main types of stones are encountered in clinical practice calcium stones predominate, and a majority of these are composed of calcium oxalate. Whatever their composition, stones are organized masses of crystals that grow on the surfaces of the renal papillae whenever the excretory burden of poorly soluble materials. In the cases of calcium and cystine stones, the main causes are over excretion of calcium, uric acid, oxalate, cystine, respectively. Uric acid stones can be caused by over excretion of uric acid but an abnormally low urine pH is usually more important in pathogenesis. Struvite stones are produced

only by bacteria that possess the enzyme urease and therefore are a result of urinary infection (Schrier , 1995).

Table 1: Types of renal stones

Major constituent	Crystal type	Approximate % of all stones
Calcium	Calcium oxlate Hydroxyapatite ($CaPO_4$) Brushite	75
Uric acid	Uric acid	5
Cystine	Cystine (amino acid)	5
Struvite- carbonate	$MgNH_4PO_4$ and $CaCO_3$	20

3.4.1. Calcium stones

Hypercalciuric states, hyperuricosuria, and hyperoxaluria are the main remediable causes of calcium stones.

i. Hypercalciuria

The association between increased urinary calcium excretion and calcium oxalate renal stones has been reported and about 30-60% of patients with calcium oxalate stones have increased urinary calcium excretion in the absence of raised serum calcium levels. Urinary calcium excretion depends on dietary calcium intake, which varies between 400-2000 mg/day. Thus, the diagnosis of hypercalciuria requires a strict definition (Gupta et al., 2002).

- Excretion of greater than 200 mg of calcium/24 hrs after one week adherence to a 400 mg of calcium and 100 mg of sodium diet.
- Excretion of greater than 4 mg of calcium/kg body weight or greater than 7 mmol in men and 6 mmol in women.
- Excretion of urinary calcium of greater than 0.11 mg/100 ml of glomerular filtrate.

- For the purpose of diagnosis and management hypercalciuria is divided into three types:

a) **Absorptive hypercalciuria**

Where the primary abnormality involves the increased intestinal absorption of calcium. This is further divided into three types.

Type 1 - the intestinal hyper absorption of calcium exists irrespective of calcium restricted diet.

Type 2 - a variant where the patients exhibit increased urinary calcium excretion while on their normal diet but normal calcium excretion on a low calcium, low sodium diet.

Type 3 - a variant with renal phosphate leak causing hypo phosphatemia which leads to increased renal synthesis of 1, 25 dihydroxy caicitriol resulting in hyper absorption of calcium and the syndrome of hypercalciuria.

b) Renal hypercalciuria characterized by primary renal leak of calcium

This involves primary renal wasting of calcium with consequent reduction in serum calcium stimulating parathyroid production. The increased parathyroid results in hydroxylation of 25, hydroxy Vit D_3 to 1, 25, dihydroxy Vit D_3 increasing intestinal calcium absorption. These effects restore the serum calcium to normal at the expense of increased parathromone 1,25, dihydroxy Vit D_3.

Two factors which differentiate renal hypercalciuria from absorptive type hypercalciuria are elevated fasting urinary calcium and stimulated parathyroid function. There is a more generalized disturbance in renal tubular function with renal hypercalciuria as shown by an exaggerated natriuretic response to thiazide and exaggerated calciuric response to carbohydrate load.

c) Resorptive hypercalciuria characterized by increased bone demineralaiztion

Hypercalciuria results from excess Parathromone dependent bone resorption as well as enhanced intestinal absorption of calcium caused by Parathromone itself or by a Parathromone dependent synthesis of 1,25 dihydroxy Vit D_3. Although Parathromone causes increased tubular absorption of calcium, the increase in the filtered load of calcium overwhelms this and results in excessive urinary calcium excretion (Gupta et al., 2002).

ii. Hyperoxaluria

Hyperoxaluria is related to calcium oxalate nephrolithiasis and is usually of three types.

a) Primary hyperoxaluria

It is of two types:

Type 1- an autosomal recessive inborn error of metabolism characterised by nephrolithiasis, tissue depostion of oxalate and death from renal failure before the age 20 in untreated patients. There is increased excretion of oxalic, glycolic and glyoxalic acids due to the defect of enzyme alanine glyoxalic acid aminotransferase (AGT) in the
liver.

Type 2- a rare varient occurs due to the deficiency of hepatic enzyme D-glycerate dehydrogenase and glyoxlate reductase, which leads to increase in urinary oxalate and glycerate excretion.

Increased hepatic conversion occurs due to the pyridoxine deficiency, ethylene glycol ingestion and methoxy flurane anaesthesia.

b) Increased oxalate absorption

Conditions causing increased oxalate absorption leading to hyperoxaluria are malabsorption occurring from bowel resection, intrinsic disease or jejunoileal bypass, which leads to increased colonic permeability of oxalate as a result of exposure of colonic epithelium to bile salts; further the

unabsorbed fats bind with the calcium making the dietary oxalate free for absorption.

c) Mild metabolic hyperoxaluria

Increased urinary oxalate excretion is seen in 0.3-0.5% of patients with calcium stones. Increased dietary protein intake and altered renal excretion of oxalate have been predicted as an important cause for increased oxalate excretion (Gupta et al.,2002).

iii. Hyperuricosuria

Patients with gout or hyperuricosuria form calcium oxalate stones apart from the uric acid stones. The most important cause for hyperuricosuria is excessive purine intake. Apart from this some patients have tendency to excrete more uric acid in the urine than do the normal subjects even on purine-free diet due to the increased uric acid production from endogenous purine metabolism. Hyperuricosuria may be seen in patients with specific enzyme defects such as increased activity of 5 phosphoribosyl 1-pyrophosphate synthetase, the enzyme that initiates purine metabolism and Lesh-Nyhan syndrome, with deficiency or complete lack of hypoxanthine guanine phosphoribosyl transferase resulting in shunting of hypoxanthine to xanthine/uric acid pathway leading to hyperuricosuria and hyperuricimea. Myeloproliferative disorders such as acute leukemia are important causes in childhood (Gupta et al., 2002).

3.4.2. Struvite stones

Struvite stones are caused by urinary infections with urease producing organisms, the most common being Proteus mirabilis. Less common pathogens include Klebsiella, Enterobacter, or Pseudomonas. (E. coll is not a urease producing organism.) Urease cleaves each mole of (soluble) urea into two moles of (relatively insoluble) ammonium.

$$NH_2CONH_2+H_2O \longleftrightarrow 2NH_3+CO_2$$

$$2NH_3+H_2O \longleftrightarrow 2NH^+ + OH^-$$

$CO_2+H_2O \longleftrightarrow H^+ + HCO_3^- \longleftrightarrow H^+ + CO_3^{2-}$

As this cleavage occurs, free H^+ is bound to NH_3 to produce NH_4, yielding OH^- from water, is making urine more alkaline. Phosphate is less soluble at alkaline versus acidic pH, so phosphate precipitates onto the insoluble ammonium products, yielding magnesium ammonium phosphate. As the bacteria that produce urease remain in urine and within the stone, they continue to produce urease, and continue to cleave urea, and so large (staghorn shaped) stones may develop quite rapidly and fill the calyceal spaces of the kidney.

3.4.3. Uric acid stone

Second only to calcareous stones in prevalence, is uric acid calculi. Uric acid exists in equilibrium with urate at a pK of 5.5. As pH falls below 5.5, the concentration of dissociated uric acid greatly exceeds that of urate. Uric acid stones can result from either hyperuricosuria, acidic urine pH. or both. In the absence of hyperuricosuria, low urinary pH also can convert urinary urate into the sparingly soluble uric acid. Excessively low urine pH is much more common than hyperuricosuria as a cause of uric acid stones. Secondary causes of low pH can result from excessive acid load or alkali loss, such as arises with chronic diarrhoea.

Studies have emphasized the increasing importance of insulin resistance in the pathogenesis of uric acid stones. High body-mass index, glucose intolerance, and over type 2 diabetes are common in uric acid stone formers. Conversely, diabetic stone formers have a 30-40% rate of uric acid stones compared with the 5-8% rate of uric acid nephrolithiasis in the general stone forming population. The results of a retrospective analysis 74 of more than 4000 patients show that individuals with a high body-mass index tend to have low urinary pH. These findings link uric acid stones and excessively acidic urine to obesity and type 2 diabetes. Metabolic studies in people indicate that uric acid stone formers maintain acid-base balance, but tend to have higher acid

production of a non-dietary origin and use titratable acid rather than ammonium to excrete their acid (Orson W Moe., 2006).

3.4.4. Cystine stone

Cystine stones are caused by inherited defects of renal transport not, as suggested by the father of genetic disease, Sir Achibald Garrod, by a defect in metabolic enzymes. Incidence and prevalence rates vary greatly dependent on geographic area and method of screening, so the actual allelic frequency is difficult to estimate. However, an incidence of one per 20.000 is often quoted. Inactivating mutations in one of the two possible subunits (rBAT or bO+ATl) of the multi substrate basic amino acid transporter in the kidney leads to urinary wasting of a host of amino acids, such as cystine, arginine, lysine, and ornithine. The phenotype is cystine stones because only cystine is soluble in urine. The solubility of cystine is improved with alkaline pH and homodimerisation of cystine to cysteine. The old clinical classification is now correlated with a molecular classification: in type I cystinuria (rBAT mutations), heterozygotic carriers have concentrations of urinary cystine within the normal range; in non-type I (ie., type II and III; b0+AT mutations) intermediate aminoaciduria is seen in heterozygotes (Orson W Moe., 2006).

3.4.5. Xanthine stone

Xanthine stones are secondary to a congenital deficiency of xanthine oxidase. This enzyme normally catalyzes the oxidation of hypoxanthine to xanthine and of xanthine to uric acid. It is of interest that allopurinol, used to treat hyperuricosuric calcium nephrolithiasis and uric acid lithiasis, produces iatrogenic xanthinuria. Blood and urine levels of uric acid are lowered, hypoxanthine and xanthine levels are increased; however, there are no case reports of xanthine stone formation resulting from allopurinol treatment. It is unlikely that allopurinol completely inhibits xanthine oxidase. Approximately 25% of patients with a xanthine oxidase deficiency develop urinary stones. The stones are radiolucent and are tannish-yellow in colour. High fluid intake and

urinary alkalization are required for prophylaxis. If stones reoccur, a trial of allopurinol and a purine-restricted diet is appropriate (Smith, 1995).

3.4.6. Rare stone

In all series of stones analysed. Between 1 and 2 percent consist of a range of rare constituents derived from either some hereditary or congenital inborn error of metabolism, such as cystinuria (not to be confused with cystinosis) , xanthinuria , or 2.8-dihydroxyadeninuria , or from a prescribed drug or metabolite , which is relatively insoluble in urine .

Examples are silica (from excess ingestion of the antacid magnesium trisilicate or from the use of pectin and silicum to thicken milk for infant feeding), sulphonamides, indinavir and triamterene. All stones contain a small percentage by weight of mucoproteinaceous matrix.Some stones consist almost entirely of mucoprotein, and usually result from inflammation of the urinary tract in patients whose urine is not sufficiently supersaturated to mineralize the organic matrix (David A. Warrell, 2003).

3.5. Modifiers of crystallization

One factor that might affect the kinetics of the process involved is the presence or absence in urine of so called modifiers of crystallization, claimed to be of particular importance in the formation of calcium containing stones. One group of crystallization modifiers is said to retard the rate of growth and/or aggregation of crystals, or the binding of calcium containing crystals to cell walls. These are known as inhibitors of crystallization and include magnesium, citrate pyrophosphate, ADP, ATP, at least two phosphopeptides, glycosaminoglycans, Tamm-Horsfall protein, ephrocalcin, calgranulin, fibronectin, various plasma proteins, osteopontin (uropontin), α_1 microglobulin, β_2 microglobulin. Urinary prothrombin fragment 1, and inter- α -trypsin inhibitor. Of these, urinary citrate is probably the most important. The second group of modifiers is claimed to promote one or more of the processes

involved in crystallization. These are known as promoters of stone formation and include matrix substance A, various uncharacterized urinary proteins and glycoproteins, and polymerizes form of Tamm-Horsfall protein (uromucoid). However the clinical importance of these compounds in the pathogenesis of stone formation remains unclear (David .A. Warrell. 2003).

3.5.1. Inhibitors

Inhibitors of calcium stone formation prevent crystal growth and aggregation by coating the surface of growing calcium crystals or by complexing with calcium and oxalate (Basavaraj et al., 2007).

a) Citrate

Citric acid is a tricarboxylic acid that circulates in blood complexed to calcium, magnesium and sodium at physiological pH of 7.4. Most of the circulating citrate is derived from endogenous oxidative metabolism. It is filtered freely through the glomerulus. Approximately 75% of the filtered citrate is reabsorbed in the proximal convoluted tubule. Apart from idiopathic causes, other etiological factors of hypocitraturia are - use of drugs like acetazolamide and thiazides, renal tubular acidosis, urinary tract infection, hypokalemia, hypomagnesemia and inflammatory bowel disease.

Thiazide diuretics may induce hypocitraturia owing to hypokalemia with resultant intracellular acidosis. Hypocitraturia is a common disorder occurring in >50% of patients with nephrolithiasis. Citrate has been widely studied for its stone inhibiting action in urine and it has been found to be particularly effective against the calcium oxalate and phosphate stones. Citrate appears to alter both calcium oxalate monohydrate and calcium phosphate crystallization.

b) **Pyrophosphates**

At low concentrations, 16 mM. pyrophosphate inhibits COM crystal growth by 50%. The urinary pyrophosphate level is in the range of 20-40 mM and therefore, theoretical levels are high enough to inhibit Calcium oxalate and Calcium Phosphate crystallization. Pyrophosphate and diphosphate have shown to inhibit the precipitation of Calcium Phosphate, where as diphosphates also inhibits the growth of apatite crystals. Pyrophosphate will reduce the absorption of calcium in the intestine. Oral administration of orthophosphate has shown little benefit in prevention of stone recurrence. Conversely, patients treated in a randomised, placebo-controlled study recorded increased stone formation in the orthophosphate treated group over placebo treated subjects over a 3-year period. There is a lack of scientific evidence to support preventive role of orthophosphate.

c) Magnesium

Magnesium is the fourth most abundant mineral in the body and is largely found in bones. Dietary magnesium is absorbed in the small intestines and excreted through the kidney. Only 1% of total body magnesium circulates in blood. In a supersaturated Calcium oxalate solution 2 mmol/L magnesium reduced particle number by 50%. Magnesium can form complexes with oxalate and decreases Struvite stones. Oral intake of magnesium will decrease the oxalate absorption and urinary excretion, in a manner similar to calcium by binding to oxalate in the gut. Magnesium supplementation in subjects with magnesium deficiency increases the excretion of citrate in urine. However, there is little evidence to recommend magnesium therapy in patients with urolithiasis.

d) Inter-alpha-trypsin inhibitor family of proteins

Inter-α-inhibitor (Ial) belongs to the Kunitz-type protein superfamily, a group of proteins possessing a common structural element (kunin) and the

ability to inhibit serine proteases. Ial is a glycoprotein composed of 2 heavy chains (HC1 and HC2) and one light chain, also known as bikunin.

Bikunin circulates free in plasma and is excreted in urine where it degrades further to fragments HI14 and HI8. Bikunin, a Kunitztype protease inhibitor found in human amniotic fluid and urine, exhibits anti-inflammatory and antimetastatic functions in animals and humans. It is expressed mainly in the proximal tubules and thin descending segment near the loop of Henle. It may contribute to the regulation of crystal adhesion and retention within tubules during kidney stone formation. Furthermore, the potent inhibition of Calcium oxalate crystal growth by these proteins, coupled with the known presence of bikunin and its fragments in urine, suggested the possible existence of a relationship between Ial and Calcium oxalate stone formation.

e) Osteopontin (Uropontin)

Osteopontin (OPN) is a negatively-charged aspartic acid rich protein that inhibits growth of Calcium oxalate crystals in a supersaturated solution. Osteopontin is intimately involved in the regulation of both physiological and pathological mineralization. Osteopontin is a phosphorylated protein of wide tissue distribution that is found in association with dystrophic calcification including in the organic matrix of kidney stones. Osteopontin is synthesised within the kidney and present in the human urine at levels in excess of 100 nM.

f) Urinary prothrombin fragment 1

The blood clotting factor prothrombin is degraded into three fragments -thrombin, fragment 1 and fragment 2. Fragment 1 is excreted in urine and is named Urinary prothrombin fragment (UPTF1) and is a potent inhibitor of Calcium oxalate stone formation in vitro. The organic matrix of Calcium oxalate crystals contains UPTF1, providing evidence that links the role of blood coagulation proteins with urolithiasis. UPTF1 is an important inhibitor of Calcium oxalate crystal aggregation and adherence of crystals to renal cells. In

South Africa the incidence of urolithiasis in blacks is significantly less compared to whites. UPTF1 from the black population has a superior inhibitory activity over UPTF1 from the white population. Further studies indicate that sialylated glycoforms of UPTF1 afford protection against Cacium oxalate stone formation, possibly by coating the surface of Calcium oxalate crystals.

g) Tamm-Horsfall protein

Tamm and Horsfall isolated a mucoprotein from the human urine nearly 50 years ago, and showed that the protein was able to interact and inhibit viral haemagglutination. Tamm-Horsfall protein (THP), also known as uromucoid, is an 80-kDa glycoprotein synthesized exclusively in the thick ascending limb of the loop of Henle's loop (TAL) with exception of the macula densa. THP is the most abundant protein in the urine of normal mammals. THP production ranges from 30 to 60 mg/24 hr in humans. THP may be involved in the pathogenesis of cast nephropathy, urolithiasis, and tubulointerstitial nephritis. There is good evidence that the excessive intake of animal protein predisposes to stone disease. Much controversy exists about whether THP is a promoter or an inhibitor of crystal aggregation. Most authors believe that it is an effective inhibitor of COM crystal aggregation in solutions with high pH, low ionic strength and low concentration of divalent ions and THP. In contrast, with low pH, high concentrations of calcium, sodium, and hydrogen ions as well as low THP, inhibitory activity is lost and it may even become a promoter of aggregation.

h) Glycosaminoglycans

Glycosaminoglycans (GAGs) have been identified as one of the macromolecules present in the stone matrix, chondritin sulphate, heparin sulphate and hyaluronic acid are excreted in the urine. Recently, the main GAGs found in stone matrix were identified as heparin sulphate and hyaluronic acid. They are thought to play an important role in Calcium oxalate

crystallization. GAGs concentration in the urine is too low to decrease calcium Struvite stones. In vitro, GAGs have shown to act as inhibitors of Calcium oxalate crystal growth and crystal aggregation. However, investigators have failed demonstrate any qualitative and/ or quantitative significant difference in total excretion of GAGs between stone formers and controls (Basavaraj et al., 2007).

i) Renal lithostathine

Lithostathine is a protein of pancreatic secretion inhibiting calcium carbonate crystal growth. A protein immunologically related to lithostathine is actually present in urine of healthy subjects and in renal stones, renal lithostathine (RL). Immunocytochemistry of kidney sections localized the protein to cells of the proximal tubules and thick ascending limbs of the loop of Henle. Because of its structural and functional similarities with pancreatic lithostathine, it was called renal lithostathine. RL seems to control growth of calcium carbonate crystals. Several reports showing the presence of calcium carbonate ($CaCO_3$) in renal stones suggested that crystals of $CaC0_3$ might be present in the early steps of stone formation. Such crystals might therefore promote Calcium oxalate crystallization from supersaturated urine by providing an appropriate substrate for heterogeneous nucleation.

3.5.2. Promoters

On the cell surfaces of the kidney, cell debris, protein aggregates and other crystals may provide analogous site for nucleation. These nucleation sites may lower the Struvite stones required to initiate crystallisation and therefore promote Calcium oxalate crystallization. Strong geometric similarities between the crystals of uric acid dihydrate and COM may promote overgrowth of one on the other, a process similar to the relationship between apatite and COM. Evidence suggests that uric acid and Calcium Phosphate may promote heterogeneous nucleation. Another factor that may promote the formation and

growth of intrarenal crystals is ionic calcium. Hypercalciuria can decrease inhibitor function and lead to crystallization. Furthermore, cellular responses to newly formed crystals and factors that modulate these crystal-cell interactions could stimulate the initiation of an intrarenal stone (Basavaraj et al., 2007).

3.6. Symptoms of kidney stones

Symptoms of kidney stones include

- Colicky pain "loin to groin" Often described as "the worst pain ever experienced".
- Haematuria (blood in the urine, due to minor damage to inside wall of kidney, ureter and urethra)
- Pyuria (pus in the urine).
- Dysuria (burning on urination when passing stones (rare). More typical of infection).
- Oliguria (reduced urinary volume caused by obstruction of the bladder or urethra by stone or extremely rarely, simultaneous obstruction of both ureters by a stone).
- Abdominal distention.
- Nausea/vomiting (embryological link with intestine- stimulates the vomiting center).
- Fever and chills.
- Hydronephrosis
- Postrenal azotemia (when kidney stone blocks ureter)
- Frequency in micturation: Defined as an increase in number of voids per day (>than 5 times), but not an increase of total urine output per day (2500ml). That would be called polyuria.
- Dribbling of urine
- Loss of appetite
- Loss of weight

3.7. INVESTIGATIONS

3.7.1. Laboratory Studies

Urinalysis

Evaluate the urine for evidence of haematuria and infection. Approximately 85% of patients with urinary calculi exhibit gross or microscopic haematuria. An absence of haematuria does not rule out urinary calculi; in fact, approximately 15% of patients with urinary stones do not exhibit haematuria.

Complete blood cell count

In the context of nephrolithiasis, an elevated white blood cell count suggests renal or systemic infection. A depressed red blood cell count suggests a chronic disease state or severe ongoing haematuria.

Total volume

Patients in whom stones form should strive to achieve a urine output of more than 2 L daily in order to reduce the risk of stone formation.

Patients with cystine stones or those with resistant cases may need a daily urinary output of 3 L for adequate prophylaxis.

Urinary pH

Some stones, such as those composed of uric acid or cystine, are pH-dependent, meaning that they can form only in acidic conditions. Calcium phosphate and struvite only form when the urine pH is alkaline. Although the other parameters in the 24-hour urine usually identify patients at risk of forming these stones, pH studies can be important in monitoring these patients, in optimizing therapy with citrate supplementation, and in identifying occult stone disease in some patients.

Serum electrolytes

Estimation of creatinine, calcium, uric acid, parathyroid hormone (PTH), and phosphorus studies. These are needed to assess a patient's current renal function and to begin the assessment of metabolic risk for future stone formation.

3.7.2. Imaging studies

Plain abdominal radiography

Plain abdominal radiography (also known as a flat plate or kidney, ureter, and bladder [KUB] radiography) is useful for assessing total stone burden, as well as the size, shape, and location of urinary calculi in some patients. It is also helpful in determining the progress of the stone without the need for more expensive tests with greater radiation exposures.

Renal ultrasonography

Renal ultrasonography by itself is frequently adequate to determine the presence of a renal stone. The study is mainly used alone in pregnancy or in combination with plain abdominal radiography to determine hydronephrosis or ureteral dilation associated with an abnormal radiographic density believed to be a urinary tract calculus. Ureteral calculi, especially in the distal ureter, and stones smaller than 5 mm are not easily observed with ultrasonography.

Intravenous urography

An intravenous urography (IVU) test, also known as an intravenous pyelography (IVP), has been the standard for determining the size and location of urinary calculi up until recently. IVU provides both anatomical and functional information.

Plain renal tomography

Although largely replaced by helical CT scanning without contrast, plain renal tomography is often helpful in finding small stones in the kidneys, especially in patients who are large or obese whose bowel contents complicate observation of any renal calcifications. Plain renal tomography is also useful for determining the number of stones present in the kidneys before a stone-prevention program is instituted. This information is used to better differentiate stones formed before therapy began from those formed later. (Stuart Wolf et al., 2010).

3.8 Treatment

The treatment options are medical, stone retrieval and prophylaxis.

3.8.1. Medical Care

Depending on the result of 24 hour urine collection, there are different treatment options for different stone types. Now there is convincing evidence that by treating specific biochemical abnormalities, the recurrence rate can be reduced. The three most commonly used classes of medications for stone prevention are enlisted here

1. **Thiazide diuretics** (e.g. Hydrochlorthiazide): are used to reduce urine calcium excretion, in patients with hypercalciuria.
2. **Alkali** (e.g. Potassium citrate): are used to increase the urinary citrate excretion in ratients with hypocitriuria.
3. **Allopurinol**: is used to reduce uric acid synthesis and urinary excretion in patients which hyperuricaemia or hyperuricosuria.
4. **Sodium cellulose phosphate (SCP):** is used to restore normal calcium excretion by reducing intestinal calcium absorption. The SCP may induce hypermagnesiuria leading to increase saturation of Calcium oxalate due to reduced complexation of urinary oxalate by magnesium.

5. **Penicillamine** (Cuprimine): are often recommended if drinking more fluids does not control cystine formations.
6. **Analgesic** (Diclophenac sodium): For patients with ureteral stones expected to pass spontaneously tablets of diclophenac sodium 50 mg administered twice daily during 3-10 days,might be useful in the risk of recurrent pain.
7. **Bisphosphonates:** Decrease fasting calciuria and less marked decrease in 24-hr calciuria.
8. **Potassium phosphate**: Increase serum phosphate, increase urine phosphate and possible increase in urine pyrophosphate.
9. ***Oxalobacter formigenes*** **and other** probiotics: Decrease oxalate excretion

(Choubey Ankur, et al., 2010). Cystone - The Himalaya Drug C. remedy is also prescribed by some medical practitioners.

Table 2

Cystone Formulation

Sl. No	Herbal Drugs	Quantity Added
1.	Didymocarpus peducellata	65mg
2.	Saxifraga ligulata	49mg
3.	Rubia cordifolia	16mg
4.	Cyperus scariosus	16mg
5.	Achyranhes aspera	16mg
6.	Onosma bracteatum	16mg
7.	Vernia cinerea	16mg

3.8.2 Surgical Care

Extracorporeal shock wave lithotripsy (ESWL)

Most urinary tract calculi, which require treatment, are currently managed with this ESWL, which is the least invasive of the surgical methods

of stone remova. Unfortunately, much of the literature has exposed the weaknesses of newer-generation lithotriptors. As a result. ESWL success rates are not as good as they once were.

New lithotriptors that have two shock heads, which deliver a synchronous or asynchronous pair of shocks (possibly increasing efficacy), have attracted great interest.

The shock head delivers Shockwaves developed from an electrohydraulic. electromagnetic, or piezoelectric source. The Shockwaves are focused on the calculus, and the energy released as the shockwave impacts the stone produces fragmentation. The resulting small fragments pass in the urine. ESWL is limited somewhat by the size and location of the calculus. A stone larger than 1.5 cm in diameter or one located in the lower section of the kidney is treated less successfully.

Ureteroscopy

A small endoscope, which may be rigid, semirigid, or flexible, is passed into the bladder and up the ureter to directly visualize the stone.

Percutaneous nephrostolithotomy

Percutaneous nephrostolithotomy allows fragmentation and removal of large calculi from the kidney and ureter and is often used for the many ESWL failures. A needle, and then a wire, over which is passed a hollow sheath, is inserted directly in the kidney through the skin of the flank and effective lithotrites can be used to rapidly fragment and remove large stone volumes (Stuart Wolf et ah, 2010).

Panel: Recent advances in surgical management of kidney stones

Miniaturisation of flexible ureteroscopes

- Improved ability to access all locations, including the lower pole of the kidney, the historic stumbling block for ureteroscopic approaches

Holmium:YAG (yttrium-aluminum-garnet) laser

- Any stone, irrespective of composition, can be fragmented
- Can be introduced through the smallest calibre endoscopes

Improved ureteral access sheaths

- Allow multiple entries and exits without causing repeated trauma to the ureter

Newly designed baskets

- Allow stones to be displaced from the difficult-to-access lower pole calyx to a more accessible upper pole calyx, where they can be fragmented ureteroscopically
- Provide an efficient means to retrieve stones remote from the nephrostomy tract with a flexible endoscope, precluding the need for additional percutaneous punctures (Orson W Moe., 2006).

3.8.3. Prevention

Effective kidney stone prevention is dependent on the stone type and the identification of risk factors for stone formation. An individualized treatment plan incorporating dietary changes, supplements, and medications can be developed to help prevent the formation of new stones. Regardless of the underlying etiology of the stone disease, patients should be instructed to increase their fluid intake in order to maintain a urine output of at least 2 L/d. A high fluid intake reduces urinary saturation of stone-forming calcium salts and dilutes promoters of Calcium oxalate crystallization.

A high sodium intake increases stone risk by reducing renal tubular calcium reabsorption and increasing urinary calcium. Patients should be advised to limit their dietary sodium intake to 2000-3000 mg/d. A restriction of

animal proteins is also encouraged since animal proteins provide an acid load because of the high content of sulfur-containing amino acids.

A reduced intake of calcium leads to an increased intestinal absorption of oxalate, which itself may account for an increased risk of stone formation. Vitamin C has been implicated in stone formation because of in vivo conversion of ascorbic acid to oxalate. Therefore, a limitation of vitamin C supplementation to 500 mg/d or less is recommended.

When dietary modification is ineffective, pharmacological treatment should be initiated. (Butterweck et al., 2009).

4. DIURETICS

Diuretics are drugs that increase the excretion of Na^+ and water from the body by an action on the kidney. Their primary effect is to increase the reabsorption of Na^+ & CI^- from the filtrate, increased water loss being secondary to the increased excretion of NaCl. This can be achieved by

- A direct action on the cells of the nephron.
- Indirectly modifiying the content of the filtrate.

Since a very large proportion of the NaCl and water that passes into the tubule ion the glomerulus is reabsorbed, a small increase in reabsorption can result in a marked increase in excretion.

4.1 Loop Diuretics

Example: Furosemide

There is an increase in the excretion of Ca^{+2} & Mg^{+2} and a decreased excretion of uric acid. The effect on Na+ is beneficial in the treatment of hypocalcaemia. (Rang H. P & Dale M.M., 2003).

4.2 Thiazide Diuretics

Thiazides increase magnesium excretion, but (unlike many diuretics) they decrease calcium excretion in the urine. The hypocalciuria induced by

thiazides appears to result from decreased expression of calcium transport proteins, including the epithelial calcium channel, calbindin and the Na-Ca exchanger protein, in renal tubules. The ability of thiazides to reduce Ca^{+2} excretion is the basis for their use in the treatment of kidney stones caused by excessive calcium in the urine. (George H. Brenner., 2010).

4.3 Inducing Agents of Diuretic & Saluretic activity

In vitro methods (Gerhard Vogel H., 2002)

- Carbonic anhydrase inbition
- Patch clamp technique in kidney stones
- Perfusion of isolated kidney tubules
- Isolated kidney

In vivo methods

- Diuretic activity in rats (Lipschitz test)
- Saluretic activity in rats
- Diuretic and Saluretic activity in dogs
- Clearence methods
- Micropuncture techniques in rats
- Stop flow technique

Today, medicinal plants are very important for the growth of new drugs. People are using herbal drug because of its safety, efficacy and lesser side effects. Plants and plant products have utilized with varying success to cure and prevent diseases. At present demand of natural plants derived products are increasing day by day in global countries. The significance of medicinal plants in national economy and its potential for the rapid growth of herbal products have been emphasizing frequently (Ankur et al.,2010).

The plant kingdom plays an important role in the life of humans and animals. India is the largest producer of medicinal plants and is rightly called as "Botanical garden of the world". Medicinal plants have stated to compromise about 8000 species and account for approximately about 50 %of all the higher

flowering plant species of India. In other words there are about 400 families of the flowering plants; atleast 315 are represented by India. In recent years, the use of traditional medicine information on plant research has again received considerable interest. The western use of such information has also come under increasing scrutiny and the national and indigenous rights on these resources have become acknowledged by most academic and industrial researchers. (Govind Pandey at al., 2010).

The plant *Clitoria ternatea* is commonly known as **butterfly pea** is an important medicinal plant belonging to Fabaceae family .*Clitoria ternatea* is a herbaceous plant grows as vine or creeper and flourishes most of the moist neutral soil. Flowers are solitary having blue or white colours .

Clitoria ternatea is grown as ornamental plant. In South East Asia the flowers of this pea vine are used as an ingredient to colour food item. The young or tender beans (Seed pods) are edible and are used in various medicinal preparations.

The juice of flower is reported to be used in insect bite and skin diseases. The roots are useful asthma, burning sensation, ascites, and inflammation. The entire herb is used its action on the CNS especially for boosting memory and improving intellect. The extract of the plant is used as an ingredient in Medhya- Rasayana is juvenating recipe used for the treatment of neurological disorders. The plant is considered to be good brain tonic and is useful for throat and eye infection, skin diseases, urinary troubles. Further aqueous extract of leaves have diuretic and urinary bladder diseases.

CHAPTER- II

LITERATURE REVIEW

Harbrone et.al (2000)

Antioxidants act as radical scavengers, inhibit lipid peroxidation and other free radical-mediated processes, and therefore they protect the human body from several diseases attributed to the reactions of radicals. Various phenolic antioxidants such as flavonoids, tannins, coumarins, xanthenes and, more recently, procyanidins have been shown to scavenge radicals in a dose-dependent manner and therefore are viewed as promising therapeutic drugs for free radical pathologies. Phenolic compounds are a large and diverse group of phytochemicals, which includes many different families of aromatic secondary metabolites in plants. They are known to exert various physiological effects in humans, such as inhibiting platelet aggregation, reducing the risk of coronary heart disease and cancer and preventing oxidative damage of lipid and low-density lipoprotein. Phenolic compounds have strong in vitro and in vivo antioxidant activities associated with their ability to scavenge free radicals, break radical chain reactions and chelate metals.

Many plant phenolics exhibiting antioxidant properties have been studied and proposed for protection against oxidation. Natural antioxidants occur in all parts of the plant (wood, bark, stems, pods, leaves, fruit, roots, flowers, pollen, and seeds). Flower is an important part of plant which contains a great variety of natural antioxidants, such as phenolic acids, flavonoids, anthocyanin and many other phenolic compounds.

Medicinal plants are considered as potential sources of antioxidant compounds. There is an increasing interest in the investigation of naturally occurring antioxidants from plants. One of the plants that deserve attention is *Clitoria ternatea***.** The ethanolic extract of *Clitoria ternatea* Linn, was evaluated for its in vitro antioxidant activities by DPPH free radical method. DPPH (Diphenyl picryl hydrazine) is a free radical at room temperature which

produces violet colour in ethanol. It is reduced in the presence of an antioxidant molecule, giving rise to uncoloured solution. Ascorbic acid was used as the standard drug for the determination of the antioxidant activity and the EC_{50} value of ascorbic acid was found to be 6.1 µg/ml. An increased EC_{50} value was observed (36.5µg/ml) for the plant extract when compared with standard drug ascorbic acid (6.1µg/ml). The extract exhibited potent antioxidant activity with an EC_{50} of 36.5µg/ml.

The antioxidant properties of *Clitoria ternatea* has also being assayed by using the free radical scavengers Ferric reducing power assay (FRAP), super oxide dismutase (SOD), Di phenyl picrylhydrazyl (DPPH) and total poly phenols. The study showed that methanolic extract showed good antioxidant activity than hexane and chloroform extracts.

Sarumathi K Thanarajan et. Al (2011)

The antioxidant activities of the ethanolic extract of *Clitoria ternatea* on acetaminophen (APAP) induced toxicity in rats suggest that the ethanol extract of *Clitoria ternatea* can prevent renal damage from APAP (Acetaminophen) induced nephrotoxicity in rats and it is likely to be mediated through active phytoconstituents and its antioxidant activities . Acetaminophen (APAP) is a widely used analgesic and antipyretic drug that is safely employed for a wide range of treatments Phytoconstituents like 1

Cycloprop[e]azulene,la,2,3,5,6,7,7a,7b octahydro-l, l**,** 4**,** 7 tetramethyl-, [laR-(laa,7a,7aa,7ba)] [Synonyms: Varidiflorene], Pterocarpin, 6H-Benzofuro[3,2-c] [l] benzopyran, 6a,lla-dihydro-3,9-dimethoxy-, (6aR-cis)- [Synonyms: Homopterocarpin], Isoparvifuran, Hexadecanoic acid, ethyl ester, Myo-lnositol, Propane, 1,1-diethoxy- were identified from ethanolic extract of *Clitoria ternatea* by using a gas chromatograph-mass spectrograph (GC MS). The antioxidant studies revealed that the levels of renal SOD (superoxide dismutase), CAT (catalase), GSH (reduced glutathione) and GPx (glutathione peroxidase) in the APAP treated animals increased significantly along with a

reduced MDA (malondialdehyde) content in ethanolic extract of *Clitoria ternatea* treated groups.

The white flowered leaves had higher content of all the enzymic antioxidants analyzed than the blue flower . The enzymatic antioxidant activity of *Clitoria ternatea* was analyzed by using goat liver slices, in both blue flowered leaf and white flowered leaf of *Clitoria ternatea* and H_20_2 was used as oxidant.

The total phenolic compounds (TPC) and 1, 1-dipheny l-2-picrylhydrazyl (DPPH) scavenging activity in the flowers and leaves of *Clitoria ternatea* has been analysed and the presence of antioxidant activity in both leaves and flowers showed that *Clitoria ternatea* have the potential to be an alternative source of natural antioxidants. It is concluded that scavenging activity expressed by *Clitoria ternatea* flower is affected by the amount of total phenolic compound.

Jain Sukla et.al.; (2008)

Phytochemical analysis has revealed that the stem contains phytosterols, phenolic compound, flavonoids and carbohydrates. Various in vitro models were applied to evaluate anti oxidant property of these extracts**.** In vitro studies included Free Radical Scavenging Capacity (RSC) on DPPH Radicals, Scavenging capacity for hydroxyl radicals, (by measuring the degradation of 2 - deoxyribose with OH radicals generated in Fenton reaction), scavenging capacity for super oxide radicals (NBT reduction assay, Nitro blue Tetrazolium assay) and Antioxidant using β -Carotene linoleate model system (B-CLAMS). The phytoconstituents responsible for antioxidant activity were isolated by preparative TLC method. The methanolic extract showed the maximum free radical scavenging capacity as compared to acetone extract.

Comparative evaluation of in vitro antioxidant activity of root of blue and white flowered varieties of *Clitoria ternatea* showed that methanol extracts

of blue and white flowered varieties of *Clitoria ternatea* showed a very powerful antioxidant activity in DPPH radical-scavenging assay. Methanol extracts of *Clitoria ternatea* also showed significant reductive ability as well as hydroxyl radical scavenging activity. Methanol extract of white flowered variety of *Clitoria ternatea* showed more significant antioxidant activity as compared to blue flowered variety of *Clitoria ternatea*.

Kaisoon Osiramornpun et.al (2004)

The phenolic compounds and antioxidant capacities of free and bound phenolics from 12 available The edible flowers which have long been consumed as vegetable and used as ingredients in cooking, has been investigated, *Clitoria ternatea* was one of them. Major phenolic acids identified in these analyses were gallic acid, ferulic acid and sinapic acid, while predominant flavonoids were quercetin and rutin. The soluble as well as bound fractions of edible flowers are rich sources of phenolic compounds with antioxidant, DPPH radical-scavenging activity and reducing power. DPPH radical scavenging capacity of bound phenolic fraction was found to be 17.6% in *Clitoria ternatea*, this suggests that screening edible flowers as potential sources of bioactive components with high antioxidant properties may be of interest to consumers and public health workers.

A polyherbal formulation (Rheumatone) made using five medicinal plants namely *Clitoria ternatea*, *Sida cordifolia, Cleodendron serratum*, *Bacopa monnieri, Cardiospermum Halicacabum*, does not exhibit any side effects and it has the enzymatic antioxidant activity. There was a significant reduction in the levels of Super oxide dismutase (SOD), Catalase, Peroxidase and Glutathione peroxidase (GPx) in the liver and kidney of adjuvant induced arthritic rats and there was an elevated level of Super oxide dismutase, Catalase, Peroxidase noted in the Liver and Kidney of rats that were treated with the polyherbal formulation Rheumatone compared with the toxic rats. There was a significant increase of Glutathione peroxidase in Liver and Kidney

of rats treated with Rheumatone compared with Group II rats treated with Freund's Complete Adjuvant (FCA).

Jayaka .B. Suresh et.al., (2003)

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action. Diabetes mellitus is also associated with an increased risk for developing premature atherosclerosis due to independent risk factors such as hypertriglyceridemia and hypertension . Insulin therapy and oral hypoglycemic agents offer effective glycemic control; yet, their shortcomings limit their usage. The world health organization (1980) has also recommended the evaluation of the effectiveness of plants in conditions where we lack safe modern drugs water law M.E.Wang (2002) states that Phytochemicals isolated from plant sources are used for the prevention and treatment of cancer, heart disease, diabetes mellitus and high blood pressure. Plants are reputed in the indigenous systems of medicine for the treatment of various diseases, the available literature shows that there are more than 800 plant species showing hypoglycemic activity and *Clitoria ternatea* is one of them.

Chronic administration of plant extracts (100mg/kg) for 14 days reduces the blood glucose level of the diabetes induced animals (Wistar Albino rats) as compared to diabetic control group. There was significant decrease in the blood glucose level in the 7th and 14th days of the diabetes induction, showing antidiabetic effect. The effect was comparable to that of standard antidiabetic drug Glibenclamide. Hyperglycemia was induced by intra peritoneal injection of freshly prepared aqueous solution of alloxan monohydrate. Extensive damage to theislets of langerhans and reduced dimensions ofislets were found in control animals. Restoration of normal cellular populatation and size of islets with hyperplasia were seen in extract treated groups. The partial restoration of normal cellular population and enlarged size of β-cells with hyperplasia were indicative of the antidiabetic potential of the plant. Aqueous extracts of *Clitoria*

ternatea plant showed anti- hyperglycemic activity in streptozotocin treated rats and this effect is because of increase in glucose uptake and glycogen deposition in isolated rat hemi diaphragm

Daisy P & Rajashi et.al. (2009)

Clitoria ternatea leaf and flower extracts exhibit antihyperglycaemic effect in rats with alloxan-induced diabetes mellitus. The effect of orally administered aqueous extracts (400 mg/kg body weight) of *Clitoria ternatea* leaves and flowers on serum glucose, glycosylated haemoglobin, and insulin were examined in control and extract-treated diabetic rats. The aqueous extracts of *Clitoria ternatea* leaves and flowers significantly reduced serum glucose, glycosylated haemoglobin and the activities of gluconeogenic enzyme, glucose-6- phosphatase, but increased serum insulin, liver and skeletal muscle glycogen and the activity of the glycolytic enzyme, glucokinase. For all the biochemical tests performed, the leaf extract-treated rat showed essentially the same profile as those treated with the flower extract.

The alcoholic root extract of *Clitoria ternatea* has shown significant gross impact in preventing the possible complications related to brain hippocampal area CA3 and pancreatic tissue in juvenile diabetic rat experimental models . These benefits could be due to interference of number of chemical compounds present in this extract. Encephalopathy is a major complication in juvenile diabetes mellitus which cripples the potential physiomorphological growth and development in early childhood. It is very essential to diagnose and initiate the treatment at the earliest to prevent the possible complications. The ancient medical science Ayurveda mentions number of remedies to treat cognitive dysfunctions, the herbal root of *Clitoria ternatea* plant is one among them.

Kavitha & Prema Lekshmi et.al., (2004)

Clitoria ternatea leaf extract shows the synergetic effect along with Trichosanthes dioica leaf extract on the Streptozotocin-induced diabetic rat. The ethanolic extracts of *Trichosanthes dioica* leaf and *Clitoria ternatea* leaf exhibited higher degree of antihyperglycaemic activity. With regard to the mechanisms, it cannot be excluded that *Trichosanthes dioica* leaf extract and *Clitoria ternatea* leaf extract may contain some biomolecules that may synthesize the insulin receptor to insulin or stimulate the beta cells of islets of Langerhans to release insulin which may finally lead to improvement of carbohydrate metabolizing enzymes towards the establishment of normal glucose levels. Significant and higher degree of antihyperglycaemic efficacy was reviewed with combination (200 mg/kg of *Trichosanthes dioica* leaf 200 mg/kg of *Clitoria ternatea* leaf) when compared to the extent of efficacy that was obtained with 400 mg/kg dose of individual plant extracts of *Trichosanthes dioica* leaf and *Clitoria ternatea* leaf.

HEPATOPROTECTIVE POTENTIAL OF *Clitoria ternatea*

Despite remarkable advances in modern medicine, hepatic disease remains a worldwide health problem, thus the search for new medicines is still ongoing. Hepatic cells participate in a variety of metabolic activities; therefore the development of liver protective agents is of paramount importance in the protection from liver damage. The literature has constantly shown that hepatoprotective effects are associated with plant extracts rich in antioxidants. Many compounds and extracts from plants have thus been evaluated for hepatoprotective and antioxidant effects against chemically-induced liver damage. Many studies have been done on the hepatoprotective activity of *Clitoria ternatea.*

Somania R Vadnala et.al (2011)

Ethanolic extract of leaves of *Clitoria ternatea* (EECT, 200 and 400mg/kg) was evaluated for prophylactic and therapeutic hepatoprotective

activity against carbon tetrachloride induced hepatic damage Silymarin (100 mg/kg) was used as standard drug. Hepatoprotective effect of EECT was evident in prophylactic and therapeutic groups at doses of 200 and 400 mg/kg. Histopathology of liver ascertained the effect of EECT and carbon tetrachloride on cytoarchitecture of the liver. The liver section of normal control animals indicated the presence of normal hepatic parenchyma, whereas administration of carbon tetrachloride in animals showed severe centrilobular necrosis, fatty changes, vacuolization and ballooning degeneration indicating severe damage of liver cytoarchitecture. The EECT 200 mg/kg in both prophylactic and therapeutic studies showed recovery and protection from hepatocyte degeneration, centrilobular necrosis, fatty infiltration, whereas EECT 400 mg/kg showed mild to normal cytoarchitecture that indicated the dose dependent hepatoprotection of EECT. The silymarin treated animals showed slightly altered hepatic parenchyma and uniform spread sheets of hepatocytes which indicated functional liver, on account of regenerative activity. The possible prophylactic and therapeutic hepatoprotective effect of *Clitoria ternatea* leaves was attributed due to the presence of flavonoids which contributed to its antioxidant property.

Palil et.al., (2011)

Methanolic extracts of blue and white flowered varieties of *Clitoria ternatea* have potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats . Methanol extract of white flowered variety (MEWFV) effectively control SGOT (serum glutamate oxaloacetate transaminase), SGPT (serum glutamate pyruvate transaminase) and ALP (serum alkaline phosphatase) as compared to methanol extract of blue flowered variety (MEBFV). MEWFV of *Clitoria ternatea* showed more significant hepatoprotective activity as compared to MEBFV of *Clitoria ternatea.* The possible mechanism of this activity was due to free radical-scavenging and

antioxidant activity, which may be due to the presence of phenolic compounds in the extracts.

Barik D.P.Naiks et.al

The hepatoprotective effect against paracetamol-induced liver toxicity in mice of ME (Methanol Extract) of *Clitoria ternatea* leaf was studied by monitoring the levels of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and billirubin along with histopathological analysis. The mice treated with the ME of *Clitoria ternatea* leaf (200 mg/kg) showed a significant decrease in ALT, AST, and bilirubin levels, which were all elevated in the paracetamol group; this confirmed the hepatoprotective effect of *Clitoria ternatea* leaf extract against the model hepatotoxicant paracetamol. The hepatoprotective action was likely related to its potent antioxidative actjyity.

Narayana Swamy & K.Selvi et.al.,(2005)

A polyherbal formulation named "Ayush-Liv.04" consisting of *Clitoria ternatea* leaves 20% as one of its constituents was evaluated for its hepatoprotective activity against ethanol and CCI4 induced liver damage in rats. The activities of liver marker 'enzymes in serum namely AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), ALP (Serum alkaline Phosphatase), ACP(Serum acid Phosphatase) and serum bilirubin level (total) were increased in toxic group animals. But the activities of these enzymes were significantly lowered in post-treated group of rats. This suggests antihepatotoxicity of "Ayush-Liv.04".

A.D.Taranalii and T.C. Cheeramkuzhi(2000) says that *Clitoria ternatea* extracts increases the memory and central cholinergic activity in rats.

III. SCOPE OF WORK

There are several options available in the management of ureteral stones. Treatment selection depends on stone size, location and composition, efficacy of each modality and associated morbidity, equipment available, physician skill, patient health and preference and finally costs. In many cases, the management of urolithiasis is combined surgical and medical approach using percutaneous nephrolithotomy (PCNL), extracorporeal shock wave lithotripsy (ESWL) and antibiotics.

These treatments are relatively costly, painful and require expert hands and availability of appropriate equipments. This has given rise to stimulation in the search for investigating natural resources showing antiurolithiatic activity. In spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of renal calculi. Most patients still have to undergo surgery to be rid of this painful disease. Ayurveda, an indigenous system of Indian medicine, offers vast scope for the successful treatment of urolithiasis.

Plants and other natural substances have been used as the rich source of medicine. All ancient civilizations have documented medicinal uses of plant in their own ethnobotanical texts. The list of drugs obtained from plant source is fairly extensive. Many remedies have been employed during the ages to treat urolithiasis. Most of the remedies were taken from plants and proved to be useful, though the rationale behind their use is not scientifically established except for a few plants and some proprietary composite herbal drugs.

The traditional system of Indian medicinal plants recommends several plants for the treatment of lithiasis, in this background; we decided to evaluate the antilithiatic and diuretic potential *Clitoria ternatea* (L) leaves which is

commonly used as a folk medicine for lithiasis and also for its diuretic potential.

The literature survey revealed that there are no scientific studies carried out regarding antilithiatic and diuretic activity on the liquid extract *Clitoria ternatea* (L) , hence in the present study the aqueous leaves extract *Clitoria* (L) leaves was examined for its antilithiatic & Diuretic property.

PLAN OF WORK

The present study was designed with the following steps in order to assess the antilithiatic activity and diuretic potential of *Clitoria ternatea*

- Collection
- Extraction
- Identification of AECT(Aqueous extract of *Clitoria ternatea).*
- Evaluation of antilithiatic activity
  - **a.** Selection of animals
  - **b.** Induction of lithiasis
  - **c.** Experimental design
  - **d.** Assessment of Antilithiatic activity
- Evaluation of Diuretic activity
  - **a.** Selection of animals
  - **b.** Experimental design
  - **c.** Assessment of Diuretic activity.

IV. PLANT PROFILE

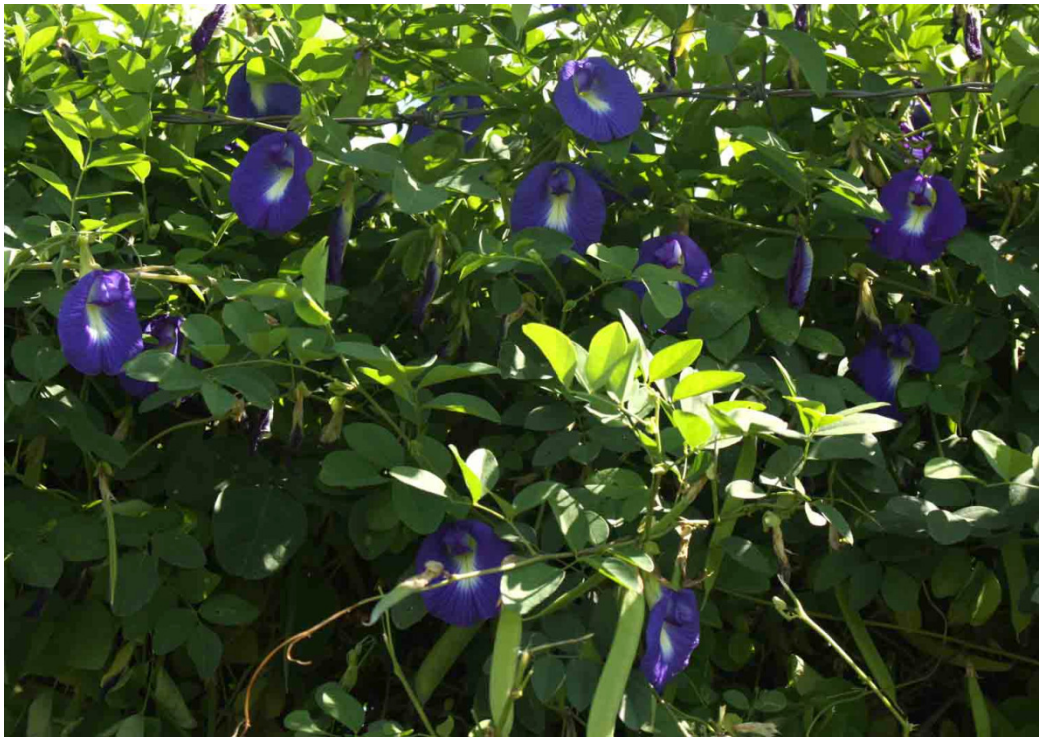


Figure 4: ***Clitoria ternatea*** L.

Figure 5: ***Clitoria ternatea*** L.

Botanical classification

- Kingdom plantae
- Subkingdom angiospermae
- Division eudicots
- Class rosids
- Order fabales
- Family fabaceae
- Genus clitoria
- Species ternata

Vernacular names

Common name: butterfly pea,blue pea,cordan pea.

- English Butterfly pea
- Brazil: Cunha.
- Sanskrit abragitha, krikarnika
- Telugu klakarnika
- Kannada sangu puspha
- Malayalam kakkattan
- Tamil sangu pusphapam

CHAPTER IV

INTRODUCTION

Clitoria ternatea (Family-•Fabaceae, previously known as Papillioneceae), a perennial twining herb, stems terete, more or less pubscent. Leaves imperipinnate, petioles 2-2.5 cm long; stipules 4 mm long, linear, acute. Leaflets 5-7, subcoriaceous, 2.5-5 by 2-3.2 cm, elliptic-oblong, obtuse or caute; stipules filiform..Flowers- axillary, solitary, standard bright or blue or sometimes white, with an orange centre; seed- 6-10, yellowish brown, smooth. Two types- white variety and blue flowered variety; widely distributed throughout Bangladesh, used as ornamental plant

Distribution

Originated from tropical Asia and later was distributed widely in South and Central America, East and West Indies, China and India, where it has become naturalized. Native to the island of Ternate in the Molluca archipelago**,** this species is now widely grown as ornamental, fodder or medicinal plant. It is found commonly as an escape in hedges and thickets throughout India to an altitude of 15cm and in Andaman Islands It can be grown as a forage legume either alone or with perennial fodder grasses in Punjab, Rajasthan, Uttar Pradesh, Gujarat, Maharashtra, Madhya-Pradesh, Andhra-Pradesh and Karnataka. The plant is also suitable as a green manure and cover crop. Besides suppressing many perennial weeds, it enriches the soil by fixing nitrogen. *Clitoria ternatea* is now widely distributed throughout the humid, low land tropics, occurring both naturally and in cultivations .

Clitoria ternatea is now widely distributed throughout the humid, lowland tropics occurring both naturally and in cultivations.

Cultivation

Clitoria ternatea is a deep-rooted, tall slender, climbing legume with five leaflets and a deep blue flower. It is well adapted to a variety of soil types (pH 5.5-8.9) including calcareous soils. It is surviving in both the extended

rainfall regions and prolonged periods of drought. Propagation is done through seed. It exhibits excellent re-growth after cutting or grazing within short period and produce high yields also. *Clitoria ternatea* L is well adapted to heavy cracking clay soils in northern Australia.

It is also used as a cover crop and green manure. The seeds are normally sown from the beginning until the middle of the wet season. It persists best when grazed lightly during the wet season .

Chemical constituent

Ethanol extract of *Clitoria ternatea* shows presence of terpenoid, flavonoid, tannin and steroid which may act as antioxidant principal. The major phytoconstituents found in Clitoria ternotea are the pentacyclic triterpenoids such as taraxerol and taraxerone. Phytochemical screening of the roots shows the presence of ternatins, alkaloids, flavonoids, saponins, tannins, carbohydrates, proteins, resins, starch, taraxerol and taraxerone.

A wide range of secondary metabolites including triterpenoids, flavones glycosides, anthocyanins and steroids has been isolated from *Clitoria ternatea* Linn. Four kaempferol glycosides I, II, III and IV were isolated from the leaves of Clitoria ternatea L. Kaempferol-3-glucoside (I), kaempferol- 3- rutinoside (II) and kaempferol-3- neohesperidoside (III) were identified by Ultra Violet, Protein Magnetic Resonance and Mass Spectrometry. (IV), C33H40O19, mp: 198, was characterized as Kaempferol-3- orhamnosyl glucoside from spectral data and was named clitorin

The seeds contain nucleoprotein with its amino-acid sequence similar to insulin, delphinidin-3,3,5-triglucoside, essential amino-acids, pentosan, watersoluble mucilage, adenosine, an anthoxanthin glucoside, greenish yellow fixed oil, a phenol glycoside, 3, 5, 7, 4-tetrahydroxy-flavone-3-rhamoglycoside, an alkaloid, ethyl D-galactopyranoside, p-hydroxycinnamic acid polypeptide, a highly basic protein-finotin, a bitter acid resin, tannic acid, 6% ash and a toxic

alkaloid. Seeds contain sitosterol, β-sitosterol, and hexacosanol and anthocyanin glucoside.

It also contains anti-fungal proteins and has been shown to be homologous to plant defensins.Aabgeena et al. reported a lectin present in the seeds of *Clitoria ternatea* agglutinated trypsin-treated human B erythrocytes. Since the purified lectin was found to be potential tool for cancer studies so an attempt was made for the alternate high yielding purification method for *Clitoria ternatea* lectin designated CTL, present in the seeds of this member of fabaceae family.

Recent study showed that malonylated flavonol glycosides were isolated from the petals of *Clitoria ternatea* with different petal colors using LC/MS/MS. It was also reported that five new anthocyanins, ternatins A3, B3, B4, B2 and D2 were isolated from *Clitoria ternatea* flowers,Kaempferol-3-glucoside,kaempferol-3-robinobiosiderhamnoside, quercetin and quercetin 3-glucoside. Six ternatins Al, A2, Bl, B2, Dl and D2 in *Clitoria ternatea* flowers were isolated by reversed phase High Performance Liquid Chromatography and their structures were partly characterized as highly acylated delphinidin derivatives. *Clitoria ternatea* was powdered and evaluated quantitatively for the analysis of total soluble sugars, protein, phenol, starch, carbohydrate and lipid .

Leaves

Leaves contain 3 monoglucoside, 3-rutinoside, 3 neohisperidoside, 3- o-rhamnosyl Glycoside, kaempferol- 3- o-rhamnosyl, aparajitin, beta-sitosterol, and essential oil.

Flower

Flower contains delphinidin-3, 5-diglucoside, delphinidin-3, 5-glucoside, and malvidin- 3β - glucoside, kaemphferol, p-coumaric acid.

Root

Contains β- carotene, stigmast- 4- ene- 3, 6 diene, taraxerol & teraxerone, starch, tannins & resirvs

Toxicity

Ethanol extract did not show any sign of toxicity upto 2000mg/kg dose .

Pharmacology

- Seeds are cathartic and the root diuretic.

Flower: Ethanol extract is used as antidiabetic.

Anxiolytic Activities

In study, the effect of alcoholic extract of aerial parts of *Clitoria ternatea* on spatial discrimination in rats followed by oral treatment with alcoholic extract at a dose of 460 mg/kg significantly prolonged the time taken to traverse the maze, which was equivalent to that produced by chlorpromazine.

The oral administration of *Clitoria* (100-400mg/kg) dose dependently increased the time spent in the open arm; the time spent in the lit box and decreased the duration of time spent in the dark box. The oral administration of *Clitoria ternatea* (30mg/kg) failed to show any significant effect in both animal models of anxiety. The animals treated with *Clitoria ternatea* (lOOmg/kg) showed a significant increase in the inflexion ratio and discrimination index which providesevidence for the species nootropic activity .

CNS Depressant Activity Studies

The *Clitoria ternatea* extract was found to possess nootropic, anxiolytic, antidepressant and anti-stress activities. The nootropic drugs facilitate intellectual performance, learning and memory.

Anti-Stress Activities of *Clitoria ternatea*

The anti-stress activity of aerial parts was assessed using cold restraint stress (CRS) induced ulcers, lithium-induced head twitches, clonidine-induced hypothermia, sodium nitrite-induced respiratory arrest and haloperidol-induced catalepsy in rat and mice

Effect of *Clitoria ternatea* on general behavior

Ethanol extract of the root of *Clitoria ternatea* shows significant neuropharmacological activity

Immunomodulatory Effects

The plant extracts have immunomodulatory effects that strengthen the immune system.

Larvicidal Activities

The methanol extracts of *Clitoria ternatea* seed extract was effective against the larvae of all the three species with LC50 values 65.2, 154.5 and 54.4 ppm, respectively for *A.stephensi*, *A.aegypti* and *C.quinquefascitus*. *Clitoria ternatea* was showing the most promising mosquito larvicidal activity .

Proteolytic Activities

The activities of endopeptidases (hemoglobin pH 3.5 and azocasein pH 6.0), carboxypeptidase benzyloxy carbonyl (CBZ-Phe-Ala Ph 5.2), and arylamidases lysophosphatidic acid and a-N-benzoyl-L-arginine P-nitro-analide (LPA 7.0 and BAPA 7.6) were assayed in extracts of cotyledons and axis of resting and germinating seeds of *Clitoria ternatea* but the endopeptidases at pH 3.5 and the arylamidase at 7.0 were high in cotyledons. The activities of carboxypeptidase and the arylamidase increased in cotyledons

reaching a maximum at the day 9, while the endopeptidases showed an increase at the day 3 followed by a decrease. In the axial tissue the endopeptidases and carboxypeptidase activities showed an increase until the day 9 followed by a decrease and arylamidase were low. The increase of acidic endopeptidases and carboxypeptidase activities in germinating cotyledons is an indication of their participation in the degradation of the storage proteins

Antihelmintic Activities:

There are so many studies which have been reported on antihelmintic activity of *Clitoria ternatea*. It was indicated that crude alcoholic extract of *Clitoria ternatea* and its ethyl acetate and methanol fractions significantly demonstrated paralysis and also caused death of worms especially at higher concentration of 50 mg/ml, as compared to standard reference piperazine citrate. Inhibitory effect of *Clitoria ternatea* leaves on free-living nematodes was evaluated using aqueous and methanol extract. In another study, flowers, leaves, stems and roots of *Clitoria ternatea* were evaluated for antihelmintic activity on adult Indian earthworms *Pheretima posthuma*. Methanol extract of root is most potent and required very less time to paralysis and death of worms as compared to other extracts. The potency increases from flowers, leaves, stems to roots .

Diuretic Activity

The powdered form of dried whole root and ethanolic extract were evaluated for diuretic activity and only single I.V. dose of extract produce moderate increase in urinary excretion of Na, K and decrease in CI but no change in urine volume. Also, an appreciable effect was seen on oral dosing

CHAPTER V

V. MATERIALS AND METHODS

Plant materials

The leaves of *Clitoria ternatea* was collected was collected from kaniyakumari district in the month of July. Then washed then dried at 70 degree C .Then the leaves are powered and then stored in air tight container.

Drugs and chemicals.

Cystone and ethylene glycol purchased from commercial sources and all other chemicals were of analytical grade.

PREPARATION AQUEOUS EXTRACT

Powdered leaves of *Clitoria ternatea* 1500gm was kept for maceration with 2000ml of water for 24hours during successive extraction. The extract was double filtered using muslin cloth and Whatmann filter paper No:1and the extract concentrated and dried on water bath. The different concentration of aqueous extract were prepared for further study.(S.Singh et.al)

Phytochemical tests for active constituents

Identification of alkaloids

- **Mayer's reagent** : to one ml of the solution add Mayer's reagent (potassium mercuric chloride)—yellow precipitate was formed.
- **Wagner's reagent** :to one ml of the aqueous extract add one ml of Wagner's reagent (aqueous iodine solution)__a brown precipitate was formed.

Identification of glycoside

Keller killiani reaction

The dried substance was dissolved in glacial acetic acid and treat with one or two drops of ferric chloride and concentrated sulphuric acid was added. The upper layer produces green colour.

Identification of trepenoid and steroid.

To 5ml of the plant extract 0.5ml of acetic anhydride and 0.5ml of chloroform was added. Then concentrated solution of sulphuric acid was slowly and red violet colour was observed for terpenoid and green bluish colour for steroid.

Test for saponins

To test the presence of saponins frothing test was done. To the extract water was added and on warming frothing showed the presence of saponins.

Test for tannins

Approximately 5g of each portion of the extract was stirred with 10ml of distilled water on a magnetic stirrer, filtered and ferric chloride reagent added to the filtrate. A blue black, green or blue- green precipitate indicates for the presence of tannins.

Tests for flavonoids

Four milligram of each plant extracts solution was treated with 1.5ml of 50% ethanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavones.

CHAPTER VI
EVALUATION OF ANTILITHIATIC ACTIVITY

Experimental Animals

Male albino wistar rats weighing between 150-200gm were used. The animals were fed with commercial rat feed pellets (Amrut laboratory animal feed Ltd. Sangli, India) and were given water ad *libitum.* They were housed in polypropylene cages under proper humidity conditions (temperature: 25 ± 2°C) and maintained on normal 12-12 h day-night cycle. The experimental protocol and all the procedures were approved by Institutional animal ethical committee (IAEC) of Ultra College of Pharmacy (UCP/IAEC/2010/54).

Ethylene glycol induced urolithiasis model

While there are several animal models that are used to study hyperoxaluria and its consequences, the most commonly employed and simplest approach to induce hyperoxaluria is to provide ethylene glycol (EG) in an animal's drinking water (Green L.M, et al.,2005)

Ethylene glycol (0.75%v/v) induced hyperoxaluria model was used to assess the antilithiatic activity in albino rat. Ethylene glycol was prepared in water (0.75% v/v) and stored in bottles before commencement of treatment.

Ethylene glycol is reported to be renotoxic, urine parameters were estimated at the end of study to assess kidney functions. Ethylene glycol major toxicities are as a result of it being metabolized to oxic metabolite by an enzyme alcohol dehydrogenase (Hadjzadeh M. A. R et al., 2008)

Ethylene glycol is readily absorbed along the intestine and is metabolized in the liver to oxalate. When ethylene glycol is metabolized by the body it produces four toxic metabolites, they are glycoldehyde, glycolate, glycolic acid and glycoxalate. These metabolite causes tissue destruction primarily from calcium oxalate crystal deposition. Oxalic acid combines with

calcium to form calcium oxalate crystals, which deposits in the kidney (Schaldt L et al., 1998).

Experimental Design

Animals were divided into five groups containing three animals in each group. Lithiasis was induced by the administration of 0.75% ethylene glycolated water to all groups viz., Lithiatic, standard and test groups except the normal control for 28 days. The lithiatic group were not received any drug. The standard group was received antiurothiatic drug cystone (750 mg/kg) from 15th day and test groups were maintained in two regimens as curative and preventive. In curative regimen the aqueous extracts of leaves of *Clitoria ternatea* was administered from 15th day and in preventive regimen the aqueous extract of *Clitoria ternatea* was given from 1^{sl} day onwards. All groups were maintaining on commercial pellet diet for 28 day (Karadi et al., 2006).

ASSESSMENT OF ANTIUROLITHIATIC ACTIVITY

Collection and Analysis of Urine

On 14th and 28th day all animals were kept in individual metabolic cages and urine samples were collected for 24hrs in measuring cylinder. During urine collection the animals were free access of normal water but not food. The collected urine sample was analyzed for calcium, magnesium, oxalate, creatinine and phosphate using standard methods. The urinary volume of all groups were also noted (Christina et al., 2005) Calcium and magnesium were determined by colorimetric method; oxalate was determined by Hodgkinson and William's method, Phosphate using Fiske and subbarow method.

Microscopic Examination of Urine

For microscopy, 1 ml of the fresh urine sample was centrifuged at 3,000 rpm (revolutions per minute) for 10 minutes, and then 950pl of the supernatant was discarded. The crystals were identified by light electron microscope. (Jie Fan, 1999)

Serum Analysis

After the experimental period, animals will be anaesthetized with diethyl ether. Blood was collected from the tail vein in non heparinized tubes and centrifuged at 2000 rpm for 20 min to obtain serum and is analysed for creatinine, BUN (blood urea nitrogen), and uric acid (Atef M Al-Attar, 2010

EVALUATION OF DIURETIC ACTIVITY

Experimental Design

Male albino wistar rats weighing between 150-200gm were used. The animals were grouped into four of three animals each and they were fasted and deprived of food and water for 18 hours prior to the experiment. The first group received only 0.9% Nacl solution 25 ml/kg, p.o. The second group served as the standard group, received the standard drug furosemide 20 mg/kg ,p.o . Rest of the two groups received aqueous extract of leaves of *Clitoria ternatea* of 250 mg/kg and 500 mg/kg suspended in 0.9% Nacl solution.

All the animals received priming dose of 0.9% Nacl solution (25 ml/kg, p.o). (Basavaraj C. Koti et al., 2010)

Table :3

Group I Normal Control	0.9% sodium chloride solution 25ml/kg, p.o.
Group II Standard	0.9% sodium chloride solution 25 ml/kg, p.o.+ standard drug Furosemide 20 mg/kg,p.o.
Group III Dose I	0.9% sodium chloride solution 25 ml/kg,p.o+ Aqueous extract of *Clitoria ternatea*(250 mg/kg body weight, p.o)
Group IV Dose II	0.9% sodium chloride solution 25 ml/kg,p.o+ Aqueous extract of *Clitoria ternatea*(500mg/kg body weight, p.o).

ASSESSMENT OF DIURETIC ACTIVITY

Collection and Analysis of Urine

After oral administration each animal were placed in an individual metabolic cages specially designed to separate feces and urine at room temperature. The observed parameters were total urine volume for 5 hours. Na^+ K^+, and CI^- excreted in urine.

The concentration of the electrolytes in urine is expressed in terms of mmol/1 and the urine volume is expressed in ml/100g/5 hours. Na^+, K^+ concentrations were measured by Flame photometer and Cl^- concentration was estimated by titration with silver nitrate solution (N/50) using three drops of 5% potassium chromate as an indicator (Basavaraj C. Koti et al., 2010).

Statistical Analysis

The results were expressed as mean ± S.D. Difference among data were determined using one way ANOVA (software) followed by Dunnetts test as per suitability $P < 0.05$ was considered as significant, $P < 0.01$ was considered as very significant.

CHAPTER VII
RESULTS

The yield obtained from the aqueous extract of leaves of *Clitoria ternatea* was about 12.3g

i. Urinary Analysis

In the present study, chronic administration of 0.75% (v/v) ethylene glycolated water to male albino rats resulted in hyperoxaluria. On 14th day, the concentration of oxalate, and phosphate were increased in lithiatic, standard, and curative regimen when compared to normal. However, the treatment with aqueous extract of *Clitoria ternatea* in preventive regimen reduced elevated levels of these ions significantly ($p < 0.01$) when compared with lithiatic control group. Contradictorily, the excretion of magnesium and calcium levels were reduced in lithiatic, standard and curative regimen but these magnesium level were significantly increased ($p < 0.01$) in preventive regimen when compared with lithiatic control group, because the standard and curative regimen doses has not received any treatment up to 14th day. (Table No.5)

The concentrations of the various ions in the collected urine were investigated and found to fluctuate drastically after the treatment. On 28th day, the supplementation with standard (Cystone 750mg/kg) and curative and preventive regimen groups (Aqueous Extract *Clitoria ternatea* 500mg/kg) lowered the elevated levels of calcium, oxalate and phosphate when compared to lithiatic control group. In both curative and preventive regimens of aqueous extract of *Clitoria ternatea* treatment significantly decreased excretion ($p<0.01$) of calcium, oxalate, creatinine and phosphate when compared to lithiatic control group, While magnesium, one of stone inhibitor increased significantly ($p<0.0l$) than in lithiatic control.

Urine Microscopy

The microscopic examination (200X) of urine of calculi induced rats (Group II) showed abundant, large crystals of Calcium oxalate with characteristic rectangular shape (Fig. 5 A). The Standard drug Cystone treated animals showed very less or almost dissolved small crystals (Fig. 5B). On curative treatment, the aqueous extract of *Clitoria ternatea* showed better dissolution of the preformed crystals of Calcium oxalate (Fig. 5C); while, on preventive treatment, the

Aqueous extract of *Clitoria ternatea* showed better prevention of stone formation along with the dissolution of preformed stones (Fig. 5D).

Serum Analysis

The Serum Uric acid and blood urea nitrogen were markedly increased in calculi-induced animals while serum creatinine was slightly elevated in Group II indicating marked renal damage. However, aqueous extract of *Clitoria ternatea* treatment in curative (Group IV) and preventive (Group V) Regimen significantly ($p<0.01$) lowered the elevated serum levels of creatinine, uric acid and BUN (Blood Urea Nitrogen).

Diuretic Activity

Urine volume (ml), urine pH, concentration of Na^+, K^+, and CI^- electrolytes (mmol/1) in the urine were recorded. The diuretic index of 5 hr urine samples were calculated to assess the diuretic potential of aqueous extract of *Clitoria ternatea* .

The urinary volume of the standard (Furosemide) and aqueous extract of *Clitoria ternatea* (250 mg/kg and 500mg/kg) significantly increased ($p<0.01$) when compared to control group. The diuretic index (Lipschitz value) of standard drug was 5.14, the AECT 250mg/kg treatment shown 1.27 and the AECT 500mg/kg treatment shown

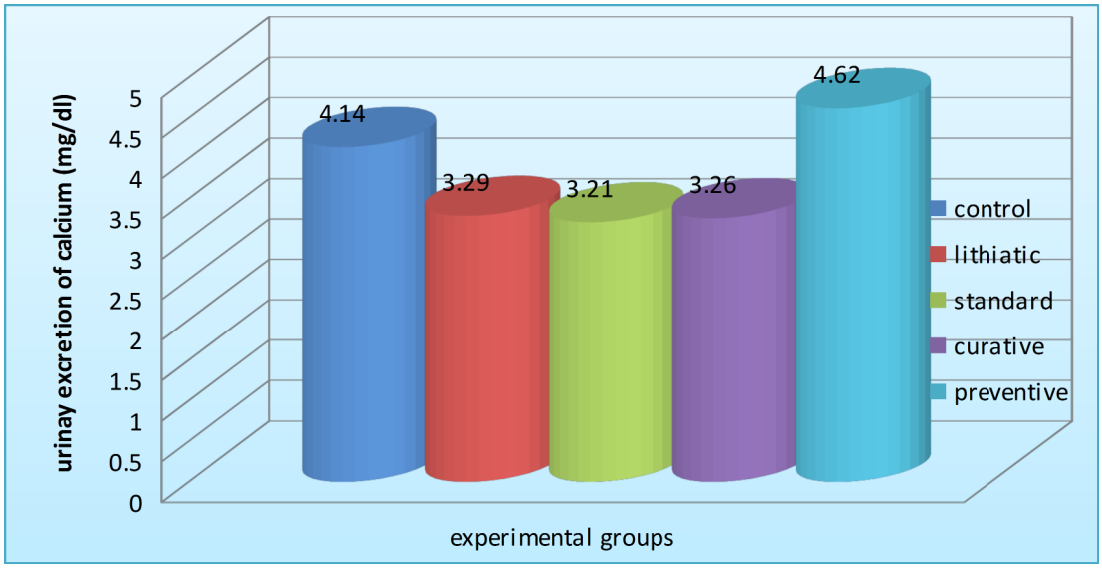
The sodium, potassium and chloride excretion of AECT treatment at both doses (250mg/kg and 500mg/kg) significantly ($p<0.01$) increased when compared to the control group. The standard drug also increased these ionic excretion levels significantly. (Table No.6)

Table.5

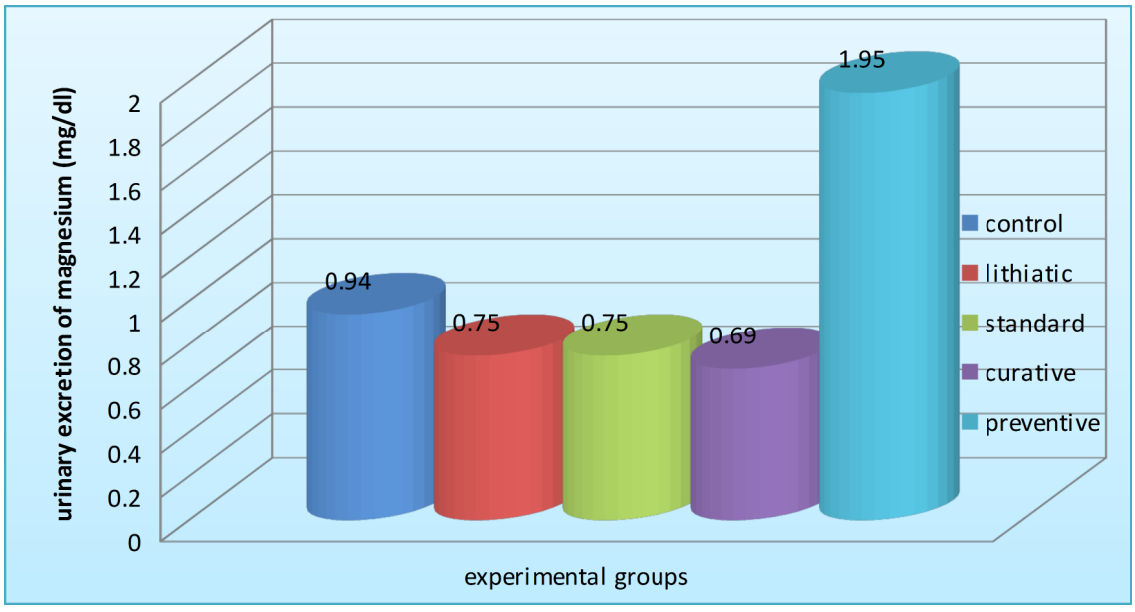
Effect of Aqueous Extract of *Clitoria ternatea* in urinary excretion of ions on 14th day

Group	Water intake (ml/24h)	Urinary Volume (ml/ 24h)	Urinary pH	Urinary Excretion (mg/dl)			
				Calcium	Magnesium	Oxalate	Phosphate
G1. (Normal Control)	7.70 ± 0.09	7.67 ± 0.105	6.40 ± 0.06	4.14 ± 0.37	0.94 ± 0.018	0.34 ± 0.027	5.85 ± 0.42
G2. (Lithiatic Control)	15.28 ± 0.33	14.96 ± 0.23	5.82 ± 0.12	3.24 ± 0.07	0.75 ± 0.04	2.82 ± 0.11	8.54 ± 0.50
G3. (Standard Treated)	15.01 ± 0.14	14.82 ± 0.17	5.77 ± 0.07	3.21 ± 0.08	0.75 ± 0.06	2.96± 0.13	8.38 ± 0.33
G4. (Curative Treatment)	15.24 ± 0.28	14.86 ± 0.13	5.79 ± 0.07	3.26 ± 0.15	0.69 ± 0.06	2.72 ± 0.08	8.23 ± 0.27
G5. (Preventive Treatment)	9.36 ± 0.19*	9.12 ± 0.06*	6.63 ± 0.06*	4.62 ± 0.07*	1.95 ± 0.02*	1.23 ± 0.12*	6.24 ± 0.26*

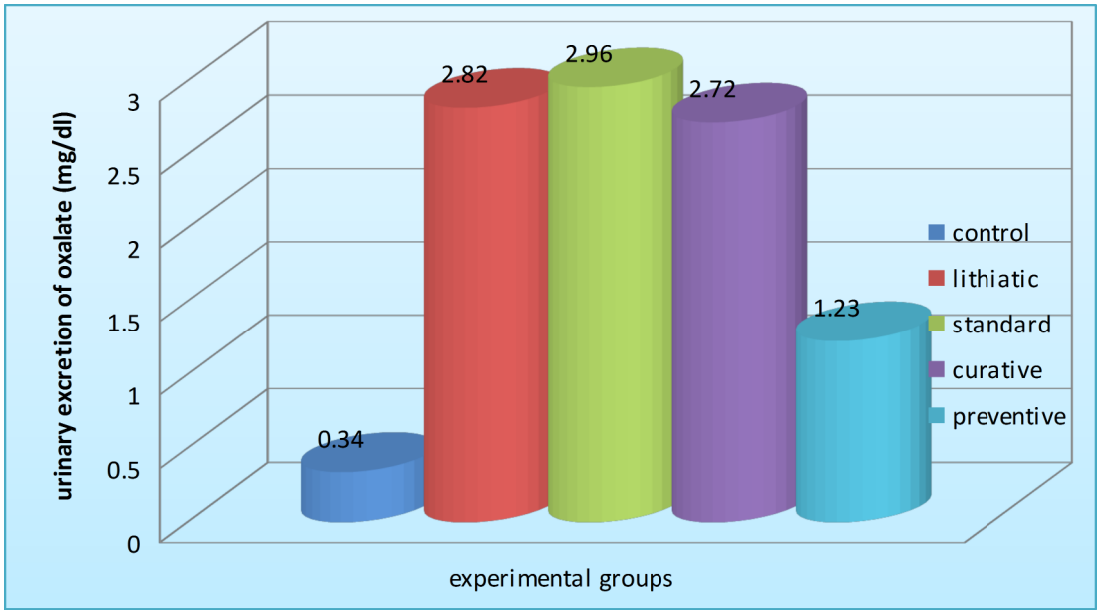
Each value expressed as the mean ± SD (n=3) one- way ANOVA; *-P<0.01 when compared with lithiatic control group.



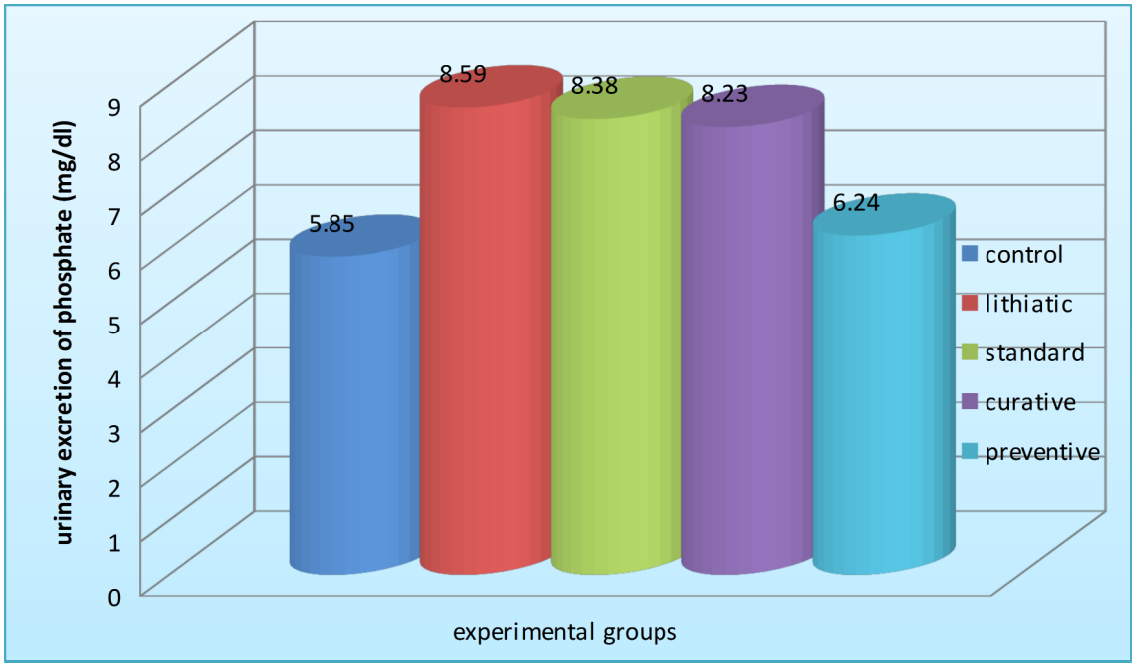
The 24 hr calcium concentration in rat's urine on day 14



The 24 hr magnesium concentration in rat's urine on day 14



The 24 hr oxalate concentration in rat's urine on day 14



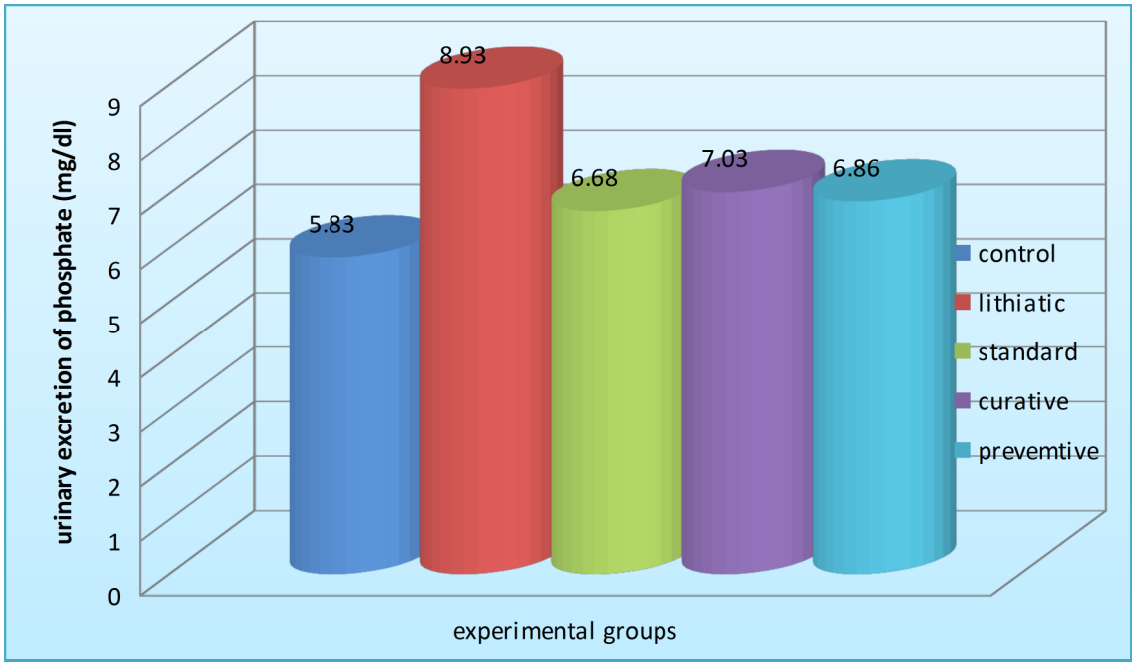
The 24 hr phosphate concentration in rat's urine on day 14

Table.6

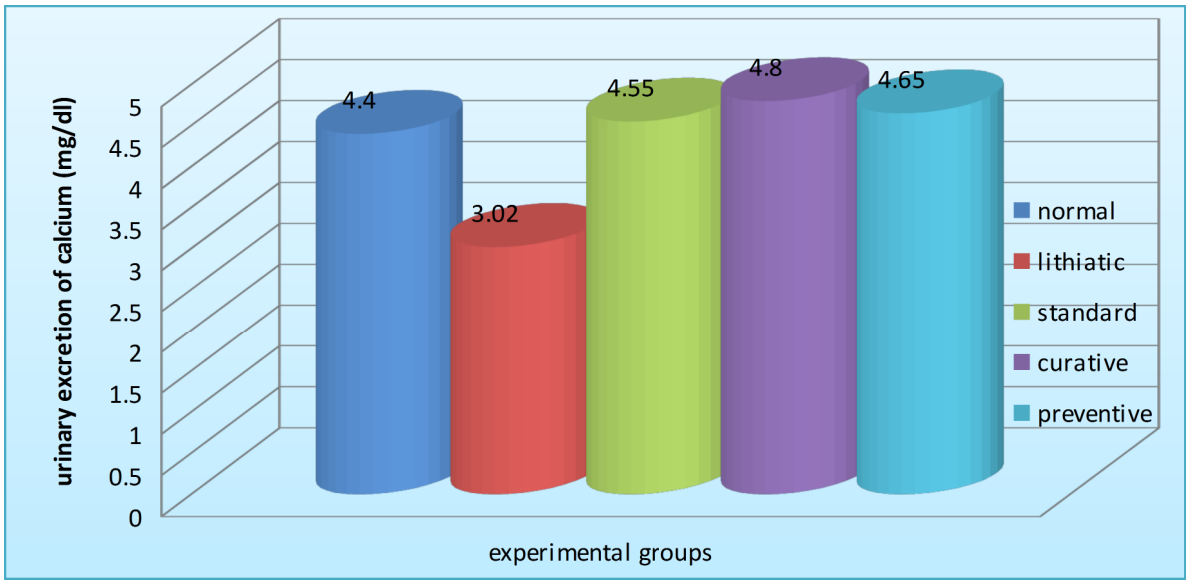
Effect of Aqueous Extract of *Clitoria ternatea* urinary excretion of ions on 28th day

Group	Water intake (ml/24h)	Urinary Volume (ml/ 24h)	Urinary pH	Urinary Excretion (mg/dl)			
				Calcium	Magnesium	Oxalate	Phosphate
G1. (Normal Control)	7.72 ± 0.10	7.89 ± 0.12	6.42 ± 0.03	4.40 ± 0.59	0.96 ± 0.59	0.34 ± 0.013	5.83 ± 0.39
G2. (Lithiatic Control)	15.62 ± 0.18ꝏ	14.26 ± 0.17⅄	5.76 ± 0.05⅄	3.02± 0.07⅄	0.55 ± 0.01⅄	3.57 ± 0.01⅄	8.93 ± 0.28⅄
G3. (Standard Treated)	10.24 ± 0.15*	9.82 ± 0.04*	6.54 ± 0.09*	4.55 ± 0.07*	1.28 ± 0.04*	1.32 ± 0.02*	6.68 ± 0.11*
G4. (Curative Treatment)	11.00 ± 0.11*	10.77 ± 0.11*	6.72 ± 0.04*	4.80 ± 0.04*	1.19 ± 0.06*	1.55 ± 0.05*	7.03 ± 0.07*
G5. (Preventive Treatment)	10.54 ± 0.20*	10.26 ± 0.20*	6.66± 0.02*	4.65 ± 0.06*	1.52 ± 0.04*	1.41 ± 0.01*	6.86 ± 0.03*

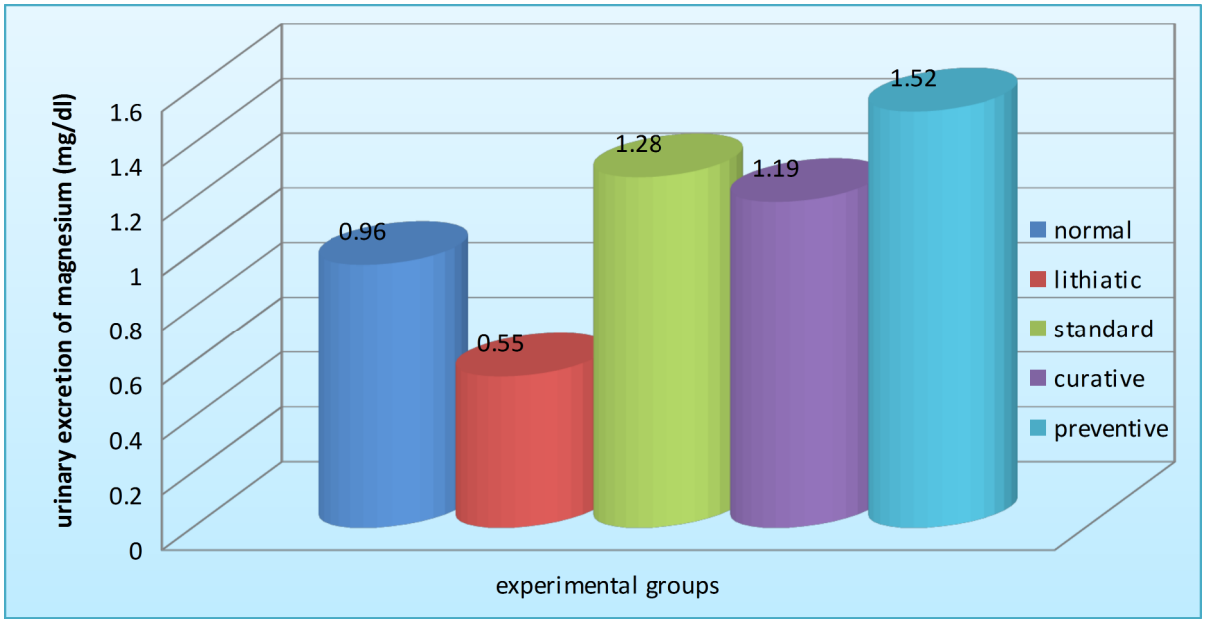
Each value expressed as the mean ± SD (n=3) one- way ANOVA; ⅄-P<0.01 when compared with the normal group; *-p<0.01 hen compared with the lithiatic group.



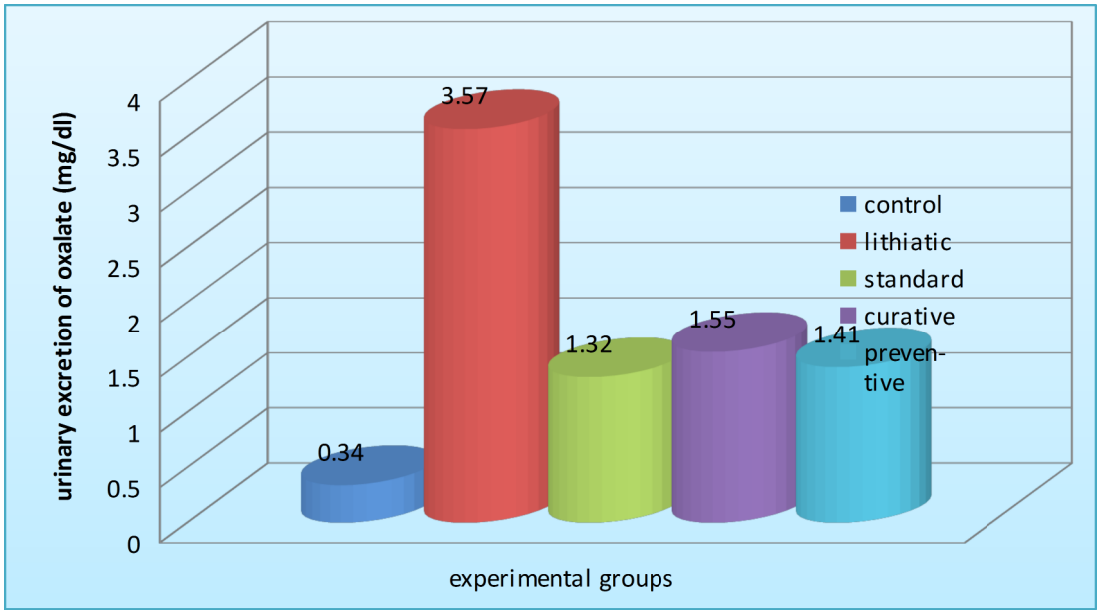
The 24 hr phosphate concentration in rat's urine on day 28



The 24 hr calcium concentration in rat's urine on day 28



The 24 hr magnesium concentration in rat's urine on day 28



The 24hr oxalate concentration in rat's urine on day 28

Table.7

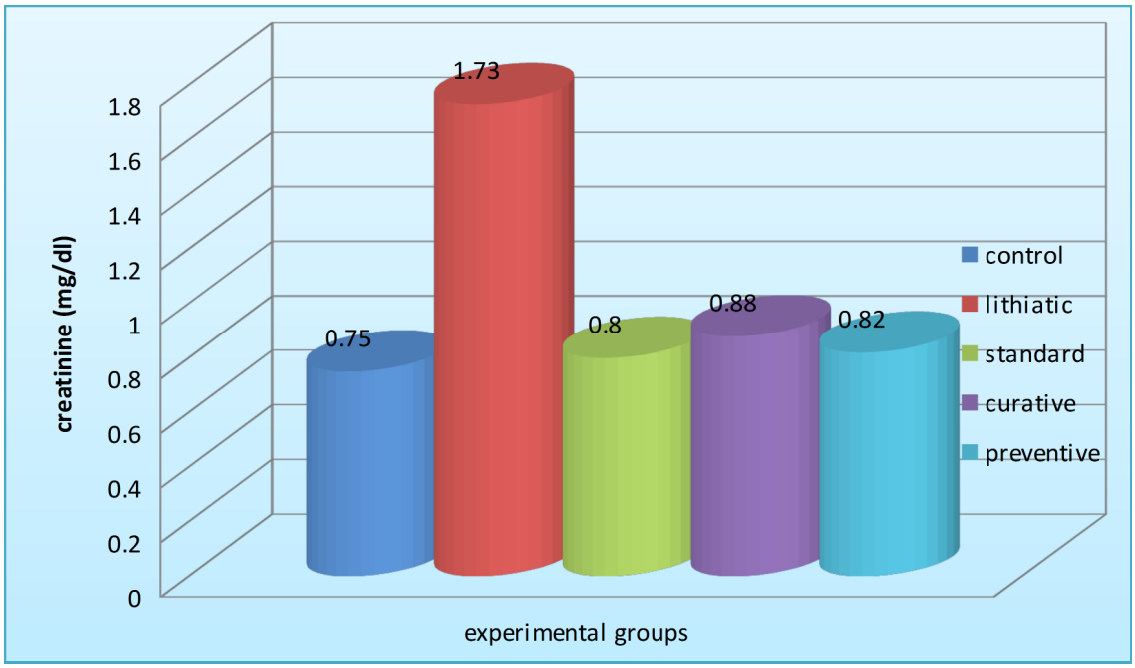
Effect of Aqueous Extract of *Clitoria ternatea* in serum parameters of lithiatic rats.

	Serum analysis (mg/d)		
	Creatinine	**Uric acid**	**BUN (Blood urea nitrogen**
Group I	0.75 ± 0.01	1.49 ± 0.07	37.61 ± 0.15
Group II	1.73 ± 0.17 ⅄	4.29 ± 0.14 ⅄	48.43 ± 0.54 ⅄
Group III	0.80 ± 0.01*	1.62 ± 0.03*	38.15 ± 1.02*
Group IV	0.88 ± 0.02*	1.92± 0.02*	40.43 ± 0.66*
Group V	0.82 ± 0.02*	1.79 ± 0.03*	39.48 ± 0.49*

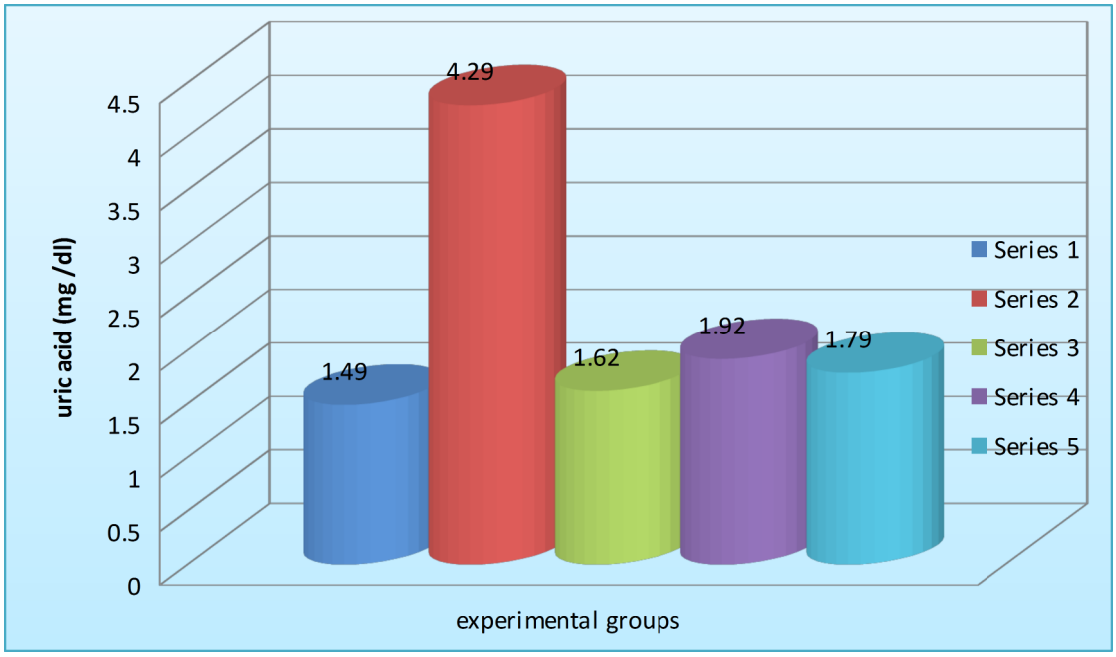
Each value expressed as the mean ± SD (N=6) one – way ANOVA ,

⅄-p<0.01 when compared with the normal group

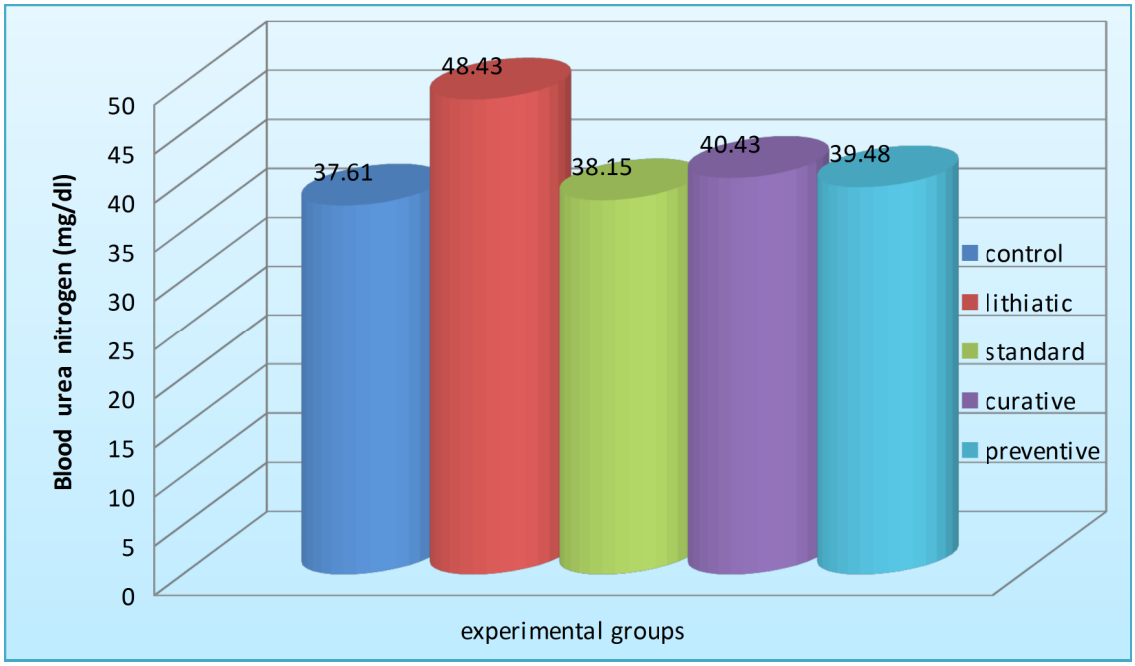
*-p<0.01 when compared with the lithiatic group



The creatinine in rat's blood on day 28



The uric acid in rats blood on day 28



The Blood urea nitrogen (BUN) in rats blood on day 28

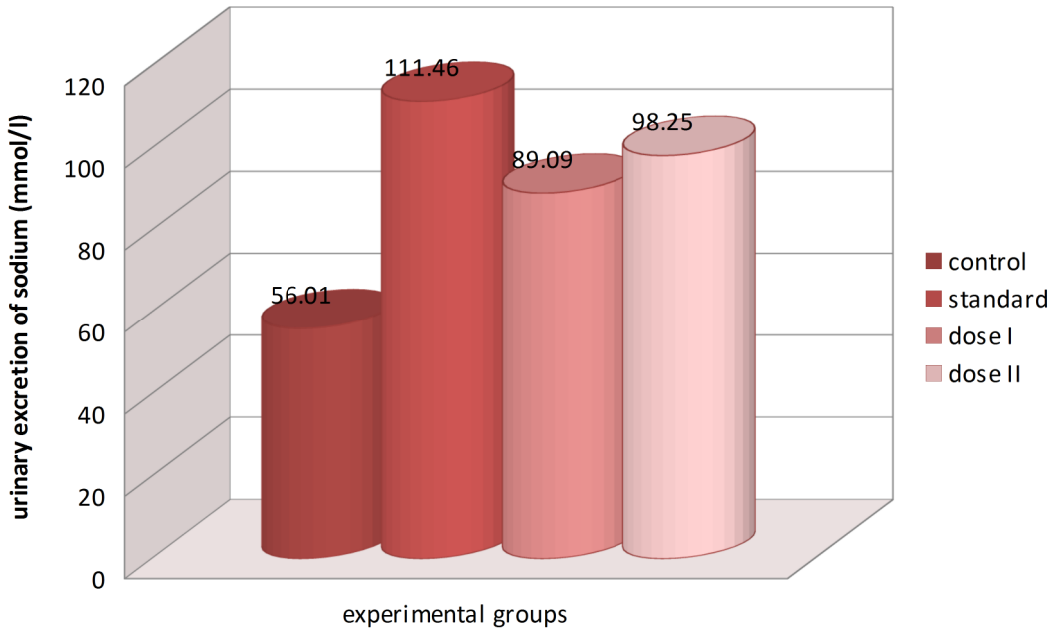
Table.8

Diuretic activity of Effect of Aqueous Extract of *Clitoria ternatea*

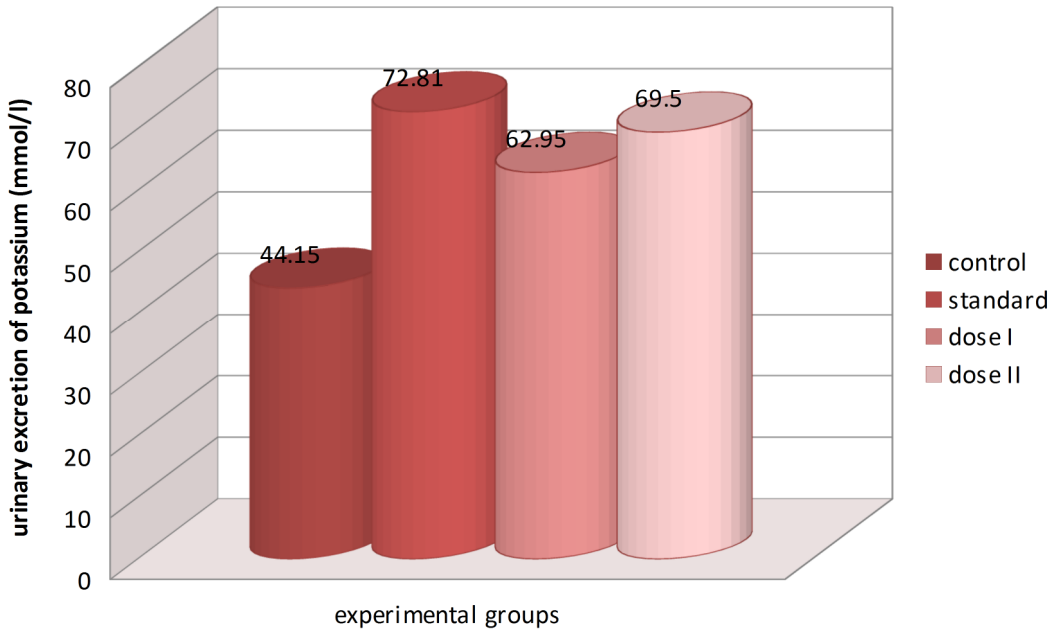
	Water intake (ml/100g//5h)	**Urine pH**	**Urinary Excretion (mg/dl)**			
			Na^+	**K^+**	**Cl^-**	**Diuretic index**
Group I	0.51 ± 0.02	6.03 ± 0.06	56.01 ± 1.53	44.15 ± 1.08	38.17 ± 4.8	-
Group II	2.88 ± 0.16	6.71 ± 0.005	111.46 ± 1.20	72.81 ± 0.14	82.20 + 0.44	5.64
Group III	1.27 ± 0.03*	6.67 ± 0.005*	89.09 ± 1.21*	62.95 ± 1.25*	58.87 ± 0.12*	2.47
Group IV	1.74 ± 0.04*	6.69 ± 0.005*	98.25 ± 0.44*	69. 50 ± 0.65*	61.16 ± 0.63*	3.41

Each value expressed as the mean ± SD(n=3)one-way ANOVA,

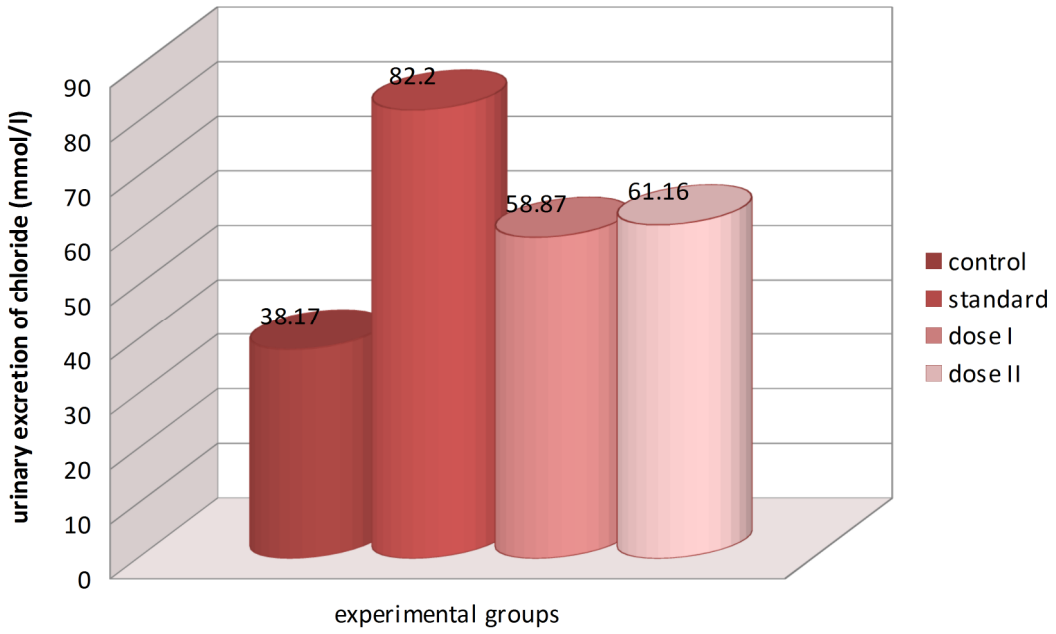
*$p<0.01$ when compared with the standard group.



The 5 hr sodium concentration in rat's urine

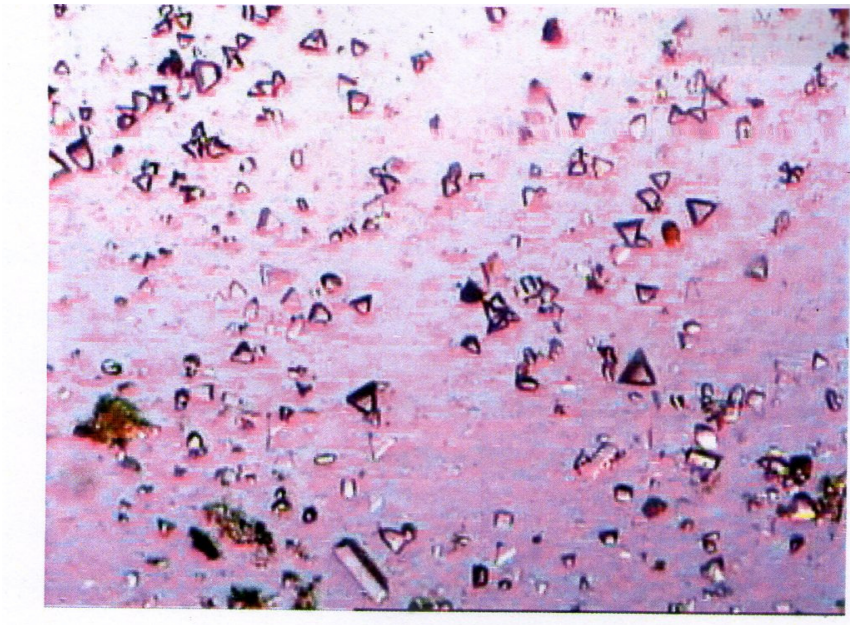


The 5hr potassium concentration in rat's urine

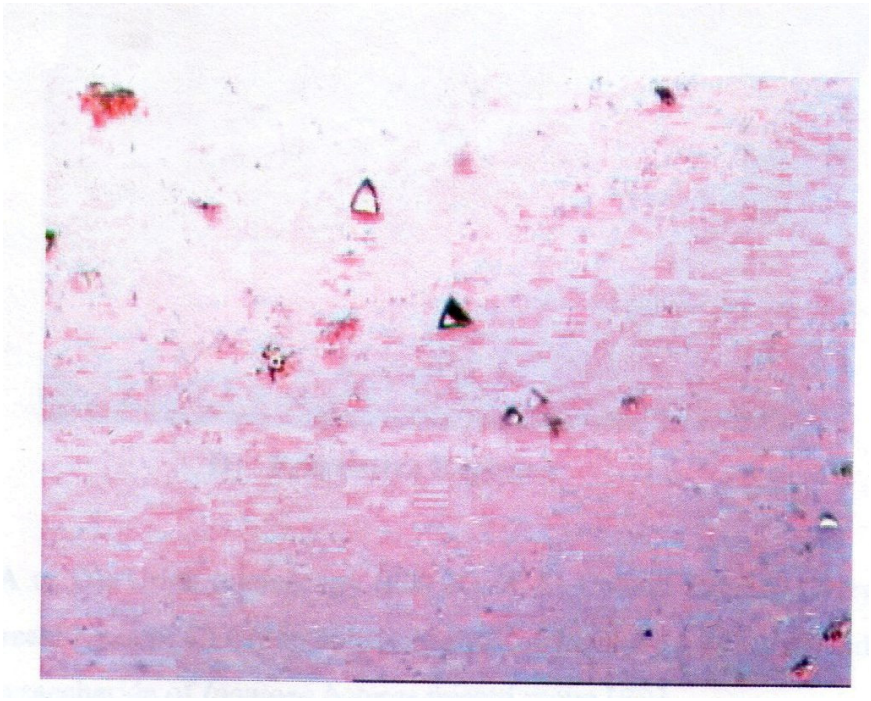


The 5 hr chloride concentration in the urine of rats

MICROSCOPIC STUDIES

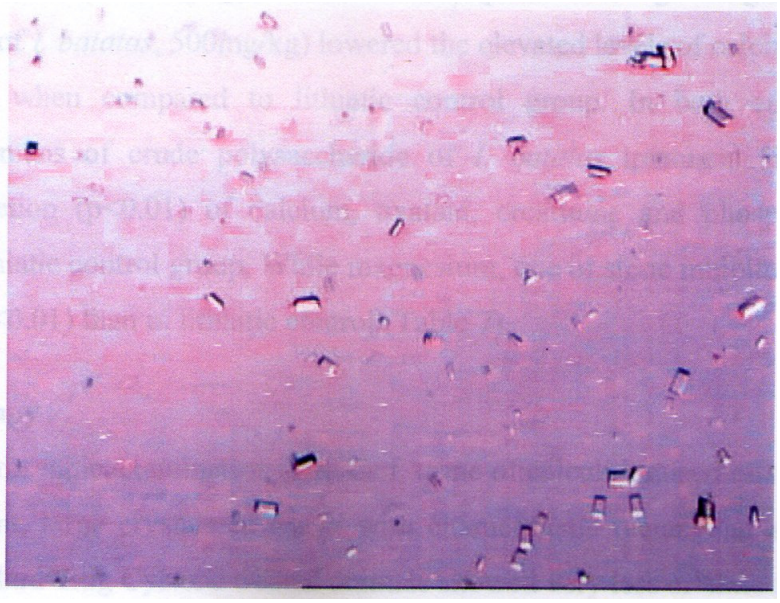


5A) Lithiatic Control



5B) Standard

5C) Curative Regimen



5D) Preventive Regimen

5.Urine Microscopy (200X) of A) Calculi induced (Untreated) group; b) Cystone treated group; C) Aqueous Extract of *Clitoria ternatea* treated group [CR]; D) Aqueous Extract of *Clitoria ternatea* treated group [PR]

CHAPTER VIII

DISCUSSION

Urinary lithiasis is generally the result of an imbalance between inhibitors & promoters in the kidneys. Human kidney stones are usually composed of Calcium oxalate crystals (Khan,et al., 2010). Rats are the most frequently used animals in Calcium oxalate deposition, as the urinary system of male rats resembles that of humans and earlier studies showed that the amount of stone deposition in female rats was significantly less (Bahuguna, et al., 2010). The urinary super saturation with stone forming constituents is generally considered to be one of the causative factors in calculogenesis (Bharathi et al., 2008).

The aqueous extract of *Clitoria ternatea* contain triterpinoids glycosides etc. it is confirmed by chemical identification test. In the present study, on 14^{th} day the oxalate, phosphate excretion was significantly ($P<0.01$) decreased in the preventive regimen when compared to lithiatic group. The calcium and magnesium excretion in the urine was significantly increased in the preventive group, where as it decreased in the lithiatic group. Urine volume and water intake was significantly increased in lithiatic control group when compared to the normal animals, whereas it was significantly ($p<0.01$) decreased in the preventive group Urinary super saturation with respect to stone forming constituents is generally considered to be one of the causative factors in calculogenesis. Administration of ethylene glycol (0.75%, v/v) to young male albino rats for 14 day period forms renal calculi composed mainly of calcium oxalate. The biochemical mechanisms for this process are related to an increase in the urinary concentration of oxalate. Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate (Sathish et al., 2010).

On 28^{lh} day, the oxalate, phosphate excretion was significantly ($p<0.01$) decreased and the calcium, magnesium excretion levels were significantly

($p<0.01$) increased in both curative and preventive regimens aqueous extract of *Clitoria ternatea* when compared with lithiatic control animals.

Following the induction of lithiasis the water intake, urinary volume and composition were found to be altered. In our study also the urinary output and waterintake was markedly increased in lithiatic control rats on day 28, however the urinary volumes of AECT and standard treated rats were significantly ($p<0.01$) decreased when compared to that lithiatic group.

In urolithiasis, the glomerular filtration rate (GFR) decreases due to the obstruction to the outflow of urine by stones in urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine, and uric acid get accumulated in blood (Bahuguna, et al., 2010). In lithiatic group, the serum levels of creatinine, uric acid, and blood urea nitrogen were elevated, while in curative and preventive groups it was decreased. The analysis of crystalluria after 28 days of treatment with stone inducing agents showed that it is markedly increased in the lithiatic group, while it is decreased in aqueous extract of leaves of *Clitoria ternatea* treatment.

In the Diuretic study, the urine volume was elevated in the AECT(250 and 500 mg/kg) treatment when compared to the control animals. The Sodium, potassium, and chloride concentrations were significantly ($P<0.01$) increased in the standard and the AECT treatment. Diuretic index is evaluated to assess the diuretic potential of the plant.

The exact mechanism of action of the plants is not known.However it may be due to following actions of the plant *Clitoria ternatea* may be responsible for the antilithiatic activity.

- By increasing using volume pH and anticalcifying activity (diuretic activity) helps in spontaneous passage

- By balancing the inhibition and promotion of crystallization in urine

- Relives the binding mucin of calculi (lithophilic activity)

- By improving renal functions, regulation of oxalate metabolism

- Regulate the crystalloid colloid imbalance and improves the renal function

- Improves renal antioxidant status and cell membrane integrity and prevent recurrence (antioxidant activity)

- Exert significant anti infective action against major curative organisms

- Reveals marked improvement in symptoms of urinary calculi like pain,burning micturation and haematuria(analgesic and anti-inflammatory) (Anubhav Nagal et.al)

However, the treatment of aqueous extract of *Clitoria ternatea* caused diuresis and hastened the process of dissolving the preformed stones and prevention of new stone formation in urinary system.

All these observations enabled us to confirm the inhibitory and curative potential, of aqueous extract of *Clitoria ternatea* on ethylene glycol induced lithiasis and its diuretic potential.

CHAPTER IX

CONCLUSION

The presented data about various ion concentrations like calcium, oxalate, magnesium, phosphate excretion in urine analysis and creatinine, uric acid, Blood urea nitrogen (BUN) in serum analysis indicates that the administration of aqueous Extract of *Clitoria ternatea* leaves to ethylene glycol induced lithiatic rats reduced and prevented the growth of urinary stones, supporting folk information regarding antilithiatic activity of the plant.

The mechanism underlying this effect is still unknown, but is apparently related to increased diuresis and lowering of urinary concentrations of stone forming constituents. These finding, thus prompt the necessity for further study to carry out the Mechanism of actions related to antilithiatic effect of Aqueous Extract of *Clitoria ternatea* leaves. By which more effective treatment for lithiasis can be achieved.

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