EVALUATION OF ANTIUROLITHIATIC ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACT OF *PHASEOLUS VULGARIS Linn SEEDS*

A Dissertation Submitted to

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

Chennai – 600 032

In Partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

VINCIYA T

Registration No: 261625252

Under the guidance of

Dr. C. VIJAYA M.Pharm., Ph.D.,

DEPARTMENT OF PHARMACOLOGY,

ULTRA COLLEGE OF PHARMACY,

4/235, COLLEGE ROAD, THASILDAR NAGAR,

MADURAI- 625020.

OCTOBER 2018

DECLARATION

I hereby declare that the Dissertation work entitled **" EVALUATION OF ANTIUROLITHIATIC ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACT OF *PHASEOLUS VULGARIS Linn* SEEDS"** submitted by me in partial fulfilment of the requirements for the award of Degree of Master of Pharmacy in Pharmacology to the Tamil Nadu Dr. M.G.R. Medical University, Chennai, work carried out at Department of Pharmacology, Ultra College of Pharmacy, Madurai during the academic year 2017-2018 under the valuable and efficient guidance of **Dr. C. Vijaya, M.Pharm., Ph.D.,** Professor, Ultra College of Pharmacy, Madurai, 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, associate ship, fellowship of any other university or institution.

Place: Madurai Reg. No: 261625252

Date: VINCIYA. T

ULTRA COLLEGE OF PHARMACY,

4/235, COLLEGE ROAD,

THASILDAR NAGAR,

MADURAI – 625020.

CERTIFICATE

This is to certify that the Dissertation work entitled "**EVALUATION OF ANTIUROLITHIATIC ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACT OF *PHASEOLUS VULGARIS Linn* SEEDS"** submitted in partial fulfillment of the requirements for the award of degree of Master of Pharmacy in Pharmacology, of the Tamil Nadu Dr. M.G.R Medical University, Chennai, is a bonafide work carried out by **VINCIYA T (Reg. No: 261625252)** guided and supervised by me during the academic year 2017-2018.

Place: Madurai

Date:

Dr. C. Vijaya, M.Pharm., Ph.D.,

Professor,

Ultra College of Pharmacy,

4/235, College Road, Thasildar Nagar,

Madurai-625020.

ULTRA COLLEGE OF PHARMACY,

4/235, COLLEGE ROAD,

THASILDAR NAGAR,

MADURAI- 625020.

CERTIFICATE

This is to certify that the Dissertation work entitled "**EVALUATION OF ANTIUROLITHIATIC ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACT OF *PHASEOLUS VULGARIS Linn* SEEDS"** submitted in partial fulfillment of the requirements for the award of degree of Master of Pharmacy in Pharmacology, of the Tamil Nadu Dr. M.G.R Medical University, Chennai, a work carried out by **VINCIYA T (Reg. No: 261625252)** guided and supervised by **Dr. C. Vijaya, M. Pharm., Ph.D.,** during the academic year 2017-2018.

Dr. P.S.S. RAMKUMAR, M. Pharm., Ph.D.,
Vice-Principal
Ultra College of Pharmacy,
4/235, College Road, Thasildar Nagar,
Madurai-625020.

Dr. A. BABU THANDAPANI , M. Pharm. Ph.D.,
Principal
Ultra College of Pharmacy,
4/235, College Road, Thasildar Nagar,
Madurai- 625020.

ULTRA COLLEGE OF PHARMACY

4/235, COLLEGE ROAD,

THASILDAR NAGAR,

MADURAI – 625020.

CERTIFICATE

This is to certify that the Dissertation work entitled "**EVALUATION OF ANTIUROLITHIATIC ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACT OF *PHASEOLUS VULGARIS Linn* SEEDS"** submitted in partial fulfillment of the requirements for the award of degree of Master of Pharmacy in Pharmacology, of the Tamil Nadu Dr. M.G.R Medical University, Chennai, a work carried out by **VINCIYA T (Reg. No: 261625252)** guided and supervised by **Dr. C. Vijaya, M. Pharm., Ph.D.,** during the academic year 2017-2018 was evaluated by us.

Place: Madurai. Examiners:

Date:

I DEDICATE THIS PROJECT TO ALMIGHTY GOD, OUR ENLIGHTING STAFFS, LOVABLE PARENTS AND MY MOTIVATING FRIENDS.

ACKNOWLEDGMENT

"Known is a drop. Unknown is an ocean."

I humbly thank the Almighty who has given me the health and ability to pass through all the difficulties in the compliance and presentation of my project.

I take privilege and honour to extent deepest gratitude and cordial thanks to my guide ***Dr. C. VIJAYA, M.Pharm, Ph.D.,*** *Professor, Ultra College of Pharmacy, Madurai for her supervision and guidance during the whole project, together with her enormous support, perpetual encouragement, abundant help and constructive criticism were the real driving force as well as the keen interest in my project encouraged me a lot, madam without your help this work not in this form. Thank you so much madam.*

With pride and pleasure, I wish to express my thanks to ***Prof. K.R. Arumugam, M.Pharm,*** *Chairman,* ***Ultra*** *College of Pharmacy, Madurai, for his encouragement and source of inspiration for my project work. I deem it my privilege in expressing fidelity Principal* ***Dr. A. Babu Dhandapani, M. Pharm, Ph.D.,*** *Ultra College of Pharmacy, Madurai, for his unswerving support and open handed help in allowing us to exploit all the facilities available in the department*

I am indebted to ***Dr. P.S.S. Ramkumar. M. Pharm, M.B.A., Ph.D.,*** *Vice Principal, Ultra College of Pharmacy, Madurai for his support and encouragement during my project work.*

A special word of thanks to ***Dr. Sampath Kumar, Ph.D.,*** *Professor, I wish to offer special thanks to* ***Mrs. Rabiyamma S.,*** *Asst. Professor, Department of Pharmacology, Ultra College of Pharmacy, Madurai without whose encouragement and guidance this project would not have got materialised.*

I wish to offer my respectable thanks to the teaching staff ***Prof.M.Chandran, Mr. V. Sivanand, Mr. Pratheesh, Mr. K. Senthil Kumar and Mr. S.K. Satheesh Kumar*** *for their valuable help and suggestions throughout my thesis work. I would like to thank* ***Mrs. G. Gomathi& Miss. Shubha madam.*** *I must record my special thanks to* ***Mrs. B. Masila,*** *B. Com, Lab technician (P.G),* ***Mrs. Pushpa,*** *D. Pharm, Lab technician (U.G), Department of Pharmacology, for her continuous assistance in carrying out the project work and special thanks to the Librarian* ***Mr.Thirunavukkarasu, M.A., M.Literauture*** *(U.G),* ***Mr.P.Sankar B.A(Lit), M.L.I.Sc (P.G)*** *and asst Librarian madam.*

I would whole heartedly thank my juniors ***Mahesh, Fayaz*** *and my seniors, for their timely support, abundant help and everlasting encouragement throughout my project.*

I offer flowers of gratitude to ***Mr. A.Thurai moni*** *(father),* ***Mrs.C.Vimala joy*** *(mother),* ***T. Veenus Babu, T. Veenus seelan, T. Veenus singh, Nihath*** *and my family members; whose inspiration, motivation, blessings and moral support continue to contribute a great deal to my academic endeavours.*

Completing task is never a one man effort. It is often result of individual contributions of a number of individuals in a direct or indirect manner.

Last but not the least, I express my gratitude and apologize to everybody whose contributions, I could not mention in this page.....

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

REVIEW OF LITERATURE

SCOPE AND PLAN OF WORK

PLANT PROFILE

MATERIAL AND METHODS

RESULT AND ANALYSIS

DISCUSSION

CONCLUSION

BIBLIOGRAPHY

1. INTRODUCTION

Formation and recurrence of kidney stones, one of the biggest challenges faced by urologists today, remains a major source of morbidity in humans. [1] This increasing urological disorder of human health affecting about 12% of the world population has been associated with an increased risk of end-stage renal failure. This growing trend is believed to be associated with changes in lifestyle modifications such as lack of physical activity and dietary habits and global warming. Recurrent stone formation is a common problem with all types of stones and therefore an important part of the medical care of patients with stone disease.[2]

In the United States, kidney stone affects 1 in 11 people, and it is estimated that 600,000 Americans suffer from urinary stones every year. In Indian population, about 12% of them are expected to have urinary stones and out of which 50% may end up with loss of kidney functions. [3] The prevalence of urolithiasis is on the rise due to various changes in the socio-demographic and other etiological factors in the north-eastern states of India in general and Manipur in particular. [4]

Stone formation in the kidney is one of the oldest and most wide spread diseases 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 1500 to1000 B.C . Reference to Stone formation is also documented in the early Sanskrit documents during 3000 and 2000 B.C. [5]

In general, urolithiasis affects all age groups from less than 1 year old to more than 70, with a male to female ratio of 2:1. The peak age for the development of calcium oxalate stones was between 50–60 years. The risk of stone formation is generally high in men; however it is becoming more common in young women. Men are at greatest risk of developing kidney stones with incidence and prevalence rates between 2–4 times that of women which could be due to the larger muscle mass of men as compared to women. This higher rate of occurrence in men than in women can also be due to enhancing capacity of testosterone and inhibiting capacity of oestrogen in stone formation . Also, the increase daily breakdown of the tissues in men could result in increased metabolic waste and a predisposition to stone formation. The other more significant cause may be because of the male urinary tract being more complicated than the female urinary tract. Estrogen may also help to prevent the formation of calcium stones by keeping urine alkaline and raising

protective citrate levels. However, recently there are reports of dramatic increase during the period from 1997 to 2002 in the prevalence of stone disease among females and a change from a 1.7:1 to 1.3:1 male to female ratio. The increasing incidence of nephrolithiasis in women might be due to lifestyle associated risk factors, such as obesity. Some reports have described that vegetarians are at lower risk for stone formation in contrast to non-vegetarians. [1].

Globally, kidney stone disease prevalence and recurrence rates are increasing, with limited options of effective drugs. Urolithiasis affects about 12% of the world population at some stage in their lifetime. It affects all ages, sexes, and races but occurs more frequently in men than in women within the age of 20–49 years. If patients do not apply metaphylaxis, the relapsing rate of secondary stone formations is estimated to be 10–23% per year, 50% in 5–10 years, and 75% in 20 years of the patient. However, life time recurrence rate is higher in males, although the incidence of nephrolithiasis is growing among females. Therefore, prophylactic management is of great importance to manage urolithiasis. Recent studies have reported that the prevalence of urolithiasis has been increasing in the past decades in both developed and developing countries. This growing trend is believed to be associated with changes in lifestyle modifications such as lack of physical activity and dietary habits and global warming. In the United States, kidney stone affects 1 in 11 people, and it is estimated that 600,000 Americans suffer from urinary stones every year. In Indian population, about 12% of them are expected to have urinary stones and out of which 50% may end up with loss of kidney functions. [3]

Studies on the geographic variation in the prevalence of kidney stone disease have shown a 50% higher prevalence in the southeast (the 'kidney stone belt') than the northwest , possibly associated with a changing state of dehydration related to high summertime temperatures and resulting in a low urine volume. Given 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. [6]

A large number of people are suffering from urinary stone problem all over the globe. Not only the humans but animals and birds also suffer from the urinary stone problem. The occurrence in some areas is so alarming that they are known as Stone Belts .Urinary stone disease is a common disorder estimated to occur in approximately 12% of the population, with a recurrence rate of 7081% in males, and 4760% in females. Approximately 50% of

patients with previous urinary calculi have a recurrence within 10 years. Stone disease is 2-3 times more common in males than in females. Most urinary calculi occur in patients aged 20 to49 years. [7]

In India, 12% of the population is expected to have urinary stones, out of which 50% may end up with loss of kidneys or renal damage. Also, nearly 15% of the population of northern India suffers from kidney stones. Fewer occurrences of urinary calculi are found in southern India, which may be due to regular dietary intake of tamarind. [7]

KIDNEY:

The urinary system is the main excretory system and consists of the following structures, 2 kidneys, 2 ureters, urinary bladder and urethra.

Definition:

Kidneys are a pair of excretory organs situated on the posterior abdominal wall, one on each side of the vertebral column, behind the peritoneum. They remove waste products of metabolism and excess of water and salt from the blood and maintain its PH.

Location:

The kidneys occupy the epigastric, hypochondriac, lumbar and umbilical regions. Vertically they extend from the upper border of twelfth thoracic vertebra to the centre of the body of third lumbar vertebra. The right kidney is slightly lower than the left, and the left kidney is little nearer to the median plane than the right.

The transpyloric plane passes through the upper part of the hilus of the right kidney and through the lower part of the hilus of the left kidney.

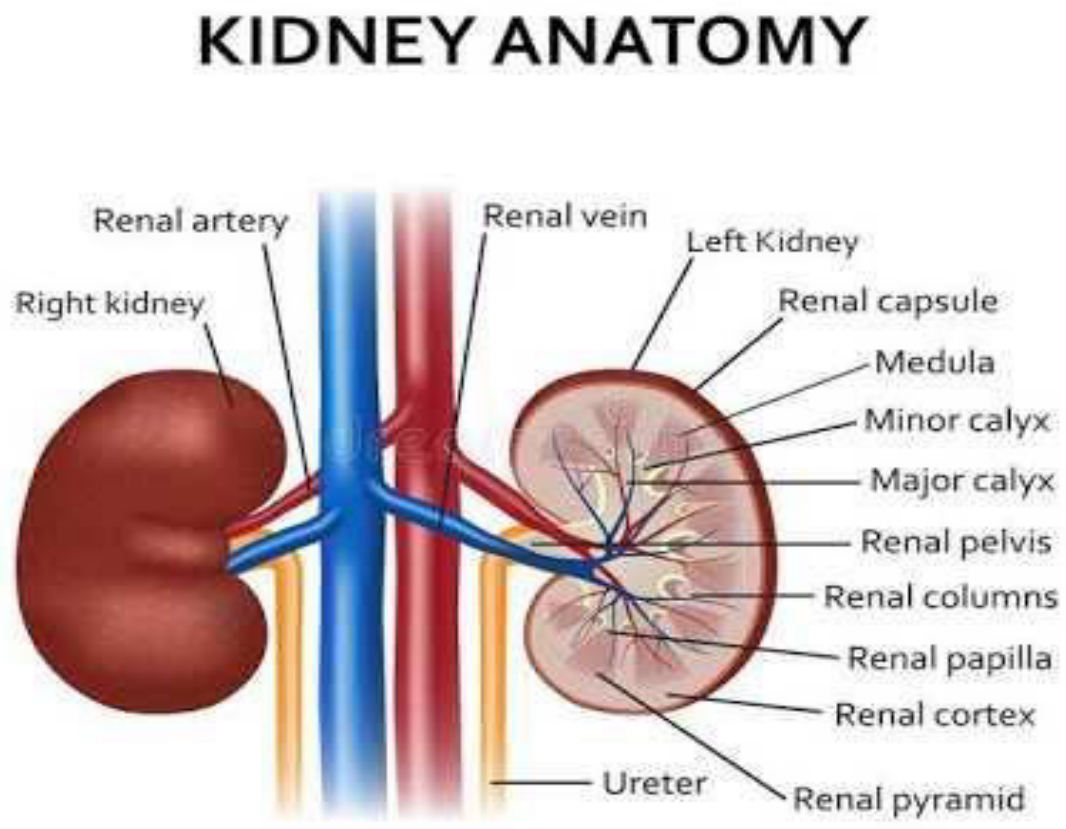


Figure No: 1 Structure of Kidney

Shape, Size, Weight and Orientation:

Each kidney is bean shaped. Each kidney is about 11cm long, 6cm broad, and 3cm thick. The left kidney is a little longer and narrower than the right kidney. On an average the kidney weighs 150 g in males and 135g in females. The kidneys are reddish brown in colour.

ANATOMY OF KIDNEY:

Longitudinal section of the kidney shows following parts.

1. Capsule: It is an outermost covering composed of fibrous tissue surrounding the kidney.
2. Cortex: It is a reddish-brown layer of tissue immediately below the capsule and outside the renal. It consists of renal corpuscles and convoluted tubules.
3. Medulla: It is the innermost layer, consisting of conical areas called the renal pyramids separated by renal columns. There are 8-18 renal pyramids in each kidney. The apex of each pyramid is called a renal papilla, and each papilla projects into a

small depression, called a minor calyx (plural calyces). Several minor calyces unite to form a major calyx. In turn, the major calyces join to form a funnel shaped structure called renal pelvis that collects urine and leads to ureter.

Blood supply to kidney:

The renal artery enters the kidney through the hilum and then branches progressively to form the interlobar arteries arcuate arteries, interlobular arteries, and afferent arterioles, which lead to the glomerular capillaries. The distal ends of the capillaries of each glomerulus combine to form the efferent arteriole, which leads to a second capillary network, the peritubular capillaries, that surrounds the renal tubules called vasa recta. The blood vessels of the venous system progressively form the interlobular vein, arcuate vein, interlobar vein, and renal vein, which leave the kidney beside the renal artery and ureter. [10],[11],[13],[14]

Functions of Kidney:

1. Endocrine functions:

Kidney is also endocrine glands. It secretes enzymes renin, 1, 25-dihydroxycholecalciferol, erythropoietin etc.

- Renin is an enzyme secreted by cells of juxtaglomerular apparatus which helps in regulation of blood pressure.
- 1, 25-dihydroxycholecalciferol; it is a biological active form of vitamin D3 found in kidney.
- Erythropoietin is essential for RBC formation.

2. Osmoregulation:

Kidney regulate osmotic pressure in the body by regulating fluids and electrolyte balance.

3. Homeostasis:

It also regulate PH balance.

4. Excretion:

- Metabolic wastes of the body are excreted in the form of urea, creatinine, uric acid etc in urine.
- Excretion of Drugs and toxins.

5. Selective reabsorption:

Glucose, amino acids, water and electrolytes etc are selectively reabsorbed in the renal tubules.

6. Erythropoiesis:

It helps in RBC formation.

7. Blood pressure regulation.

8. Secretion of prostaglandins:

The kidneys also produce prostaglandin E and prostacyclin, which have a vasodilator effect and are important in maintaining renal blood flow. [13],[14],[15]

UROLITHIASIS:

The process of forming stones in the kidney, bladder, and/or urethra (urinary tract).

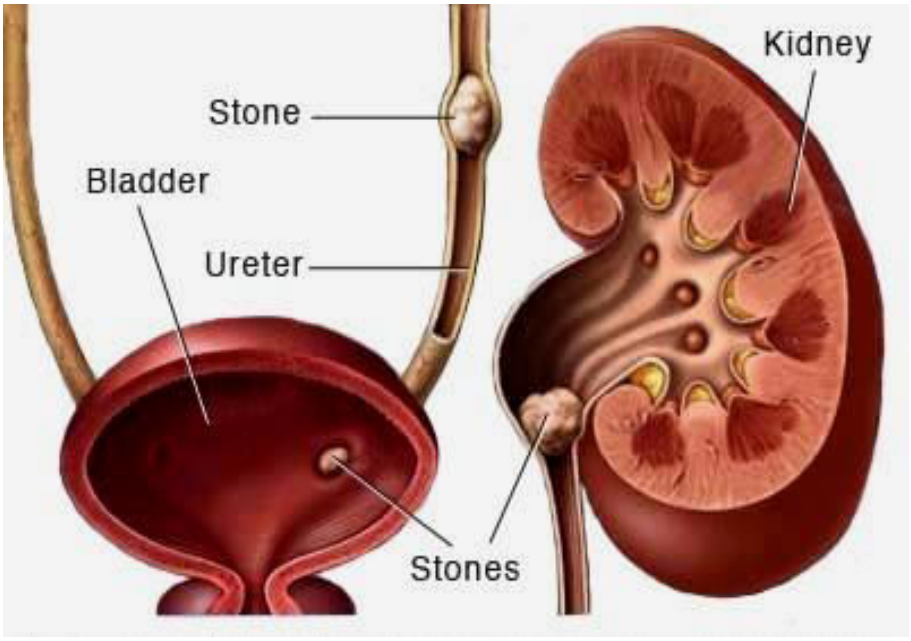


Figure No: 2 Kidney Stone

The development of stones is related to decreased urine volume or increased excretion of stone forming components such as calcium, oxalate, urate, cystine, xanthine, and phosphate. The stones form in the urine collecting area (the pelvis) of the kidney and may range in size from tiny to staghorn stones the size of the renal pelvis itself.

The pain with kidney stones is usually of sudden onset, very severe and colicky (intermittent), not improved by changes in position, radiating from the back, down the flank, and into the groin. Nausea and vomiting are common.

Factors predisposing to kidney stones include recent reduction in fluid intake, increased exercise with dehydration, medications that cause hyperuricemia (high uric acid) and a history of gout.

Treatment includes relief of pain, hydration and, if there is concurrent urinary infection, antibiotics.

The majority of stones pass spontaneously within 48 hours. However, some stones may not. There are several factors which influence the ability to pass a stone. These include the size of the person, prior stone passage, prostate enlargement, pregnancy, and the size of the stone. A 4 mm stone has an 80%chance of passage while a 5 mm stone has a 20% chance. If a stone does not pass, certain procedures (usually by a urology specialist doctor) may be needed.

The process of stone formation, urolithiasis, is also called nephrolithiasis. "Nephrolithiasis" is derived from the Greek nephros-(kidney) lithos (stone) = kidney stone

"Urolithiasis" is from the French word "urine" which, in turn , stems from the Latin "urina" and the Greek "ouron" meaning urine= urine stone. The stones themselves are also called renal calculi. The word "calculus" (plural: calculi) is the Latin word for pebble. [12],[15],[16],[17]

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 differ 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. [21]

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. [22]\

Etiology:

Several etiological factors 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 and Sex:

  The disease affected all age groups from less than 1 year old to more than 70, with 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, hyperoxaluria, hyperuricosauria, hypocitrauria 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:

Supersaturation 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 the men and 43.9% of the 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 and further concluded that 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. Urinary metabolic abnormalities such as low urine volume, hypercalciuria, hyperoxaluria, hyperuricosuria and hypocitraturia predispose a patient to early recurrence. 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 cause of calcium oxalate stones is heterogeneous 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. [21]

Types of urinary calculi:

There are 4 main types of urinary calculi-calcium containing, mixed (struvite), uric acid and cystine stones, and a few rare types.

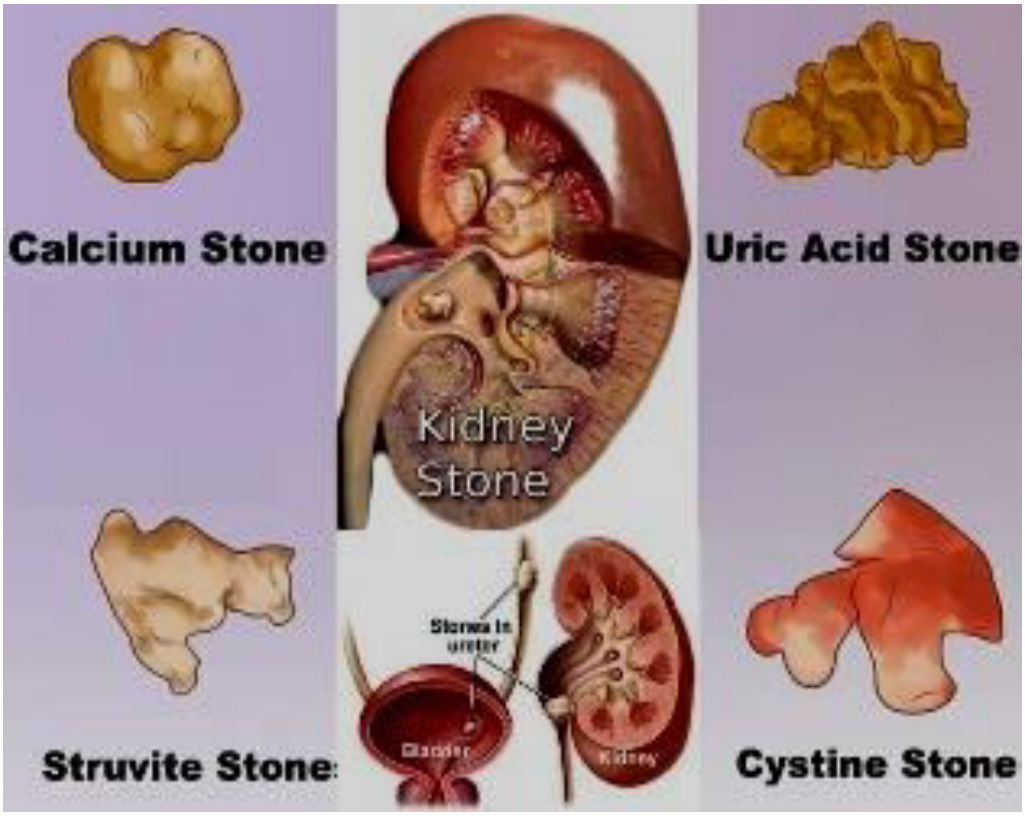


Figure No: 3 Types of Kidney Stones

1. CALCIUM STONES:

Calcium stones are the most common comprising about 75% of all urinary calculi. They may be pure stones of calcium oxalate (50%) or calcium phosphate (5%), or mixture of calcium oxalate and calcium phosphate (45%).

Etiology :

Etiology of calcium stones is variable.

About 50% of patients with calcium stones have idiopathic hypercalciuria without hypercalcaemia. Approximately 10% cases are associated with hypercalcaemia and hypercalciuria, most commonly due to hyper parathyroidism, or a defect in the bowel (i.e. absorptive hypercalciuria), or in the kidney (i.e. renal hypercalciuria).

About 15% of patients with calcium stones have hyper uricosuria with anormal blood uric acid level and without any abnormality of calcium metabolism.

In about 25% of patients with calcium stones, the cause is unknown as there is no abnormality in urinary excretion of calcium, uric acid or oxalate and is referred to as "idiopathic calcium stone disease"

Pathogenesis:

The mechanism of calcium stone formation is explained on the basis of imbalance between the degree of supersaturation of the ions forming the stone and the concentration of inhibitors in the urine. Most likely site where the crystals of calcium oxalate and or calcium phosphate are precipitated is the tubular lining or around some fragment of debris in the tubule acting as nidus of the stone. The stone grows, as more and more crystals are deposited around the nidus. Other factors contributing to formation of calcium stones are alkaline urinary pH, decreased urinary volume, and increased excretion of oxalate and uric acid.

Morphology :

Caicium stones are usually small (less than a centimetre), ovoid, hard, with granular surface. They are dark brown due to old blood pigments deposited in them as a result of repeated trauma caused to the urinary tract by these sharp edged stones.

MIXED (STRUVITE) STONES:

About 15% of urinary calculi are made of magnesium-ammonium-calcium-hosphate, often called struvite; hence mixed stones are also called as 'struvite stones' or triple phosphate stones.

Etiology:

Struvite stone are formed as a result of infection of the urinary tract with urea splitting organisms that produce urease such as by species of proteus and occasionally klebsiella, pseudomonas and enterobacter. These are, therefore, also known as infection–induced stones. However, E.coli does not form urease.

Morphology:

Struvite stones are yellow white or grey. They tend to be soft and friable and irregular in shape. Staghorn stone which is a large solitary stone that take the shape of the renal pelvis where it is often formed is an example of struvite stone.

URIC ACID STONE:

Approximately 6% of urinary calculi are made up of uric acid. Uric acid calculi are radiolucent unlike radio opaque calcium stones.

Etiology:

Uric acid stones are frequently formed in cases with hyper uricaemia and hyper uricosuria such as due to primary gout or secondary gout due to myeloproliferative disorders especially those on chemotherapy and administration of uricosuric drugs (eg salicyates, probenecid). Other factors contributing to their formation are acidic urinary pH (below 6) and low urinary volume.

Pathogenesis:

The solubility of uric acid at pH of 7 is 200 mg/dl while at pH of 5 is 15 mg /dl. Thus as the urine become more acidic, the solubility of uric acid in urine decreases and precipitation of uric acid increases favouring the formation of uric acid stones while hyper uricaemia is found in half the cases.

Morphology:

Uric acid stones are smooth, yellowish brown hard and often multiple. On cut section they show laminated structure.

CYSTINE STONES:

Cystine stones comprises less than 2% of urinary calculi.

Etiology:

Cystine stones are associated with cystinuria due to genetically determined defects in the transport of cystine and other amino acids across the cell memebrane of the renal tubules and the small intestine mucosa.

Pathogenesis:

The resultant excessive excretion of cystine which is least soluble of the naturally occurring aminoacids leads to the formation of crystals and eventually cystine calculi.

Morphology:

Cystine stones are small, rounded, smooth often multiple. They are yellowish and waxy.

OTHER CALCULI:

Less than 2% of urinary calculi consist of other rare types such as due to inherited abnormality of enzyme metabolism. E.g. hereditary xanthinuria developing xanthine stones. [13]

Symptoms of kidney stones:

- A kidney stone may not cause symptoms until it moves around within your kidney or passes into your ureter — the tube connecting the kidney and bladder. At that point, you may experience these signs and symptoms.
- Severe pain in the side and back, below the ribs
- Pain that radiates to the lower abdomen and groin
- Pain that comes in waves and fluctuates in intensity
- Pain on urination
- Pink, red or brown urine
- Cloudy or foul-smelling urine
- Nausea and vomiting
- Persistent need to urinate
- Urinating more often than usual
- Fever and chills if an infection is present
- Urinating small amounts

Pain caused by a kidney stone may change — for instance, shifting to a different location or increasing in intensity — as the stone moves through your urinary tract. [14-19]

MECHANISM OF CALCIUM OXALATE RENAL STONE FORMATION:

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 stones.

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 crystalluria 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.

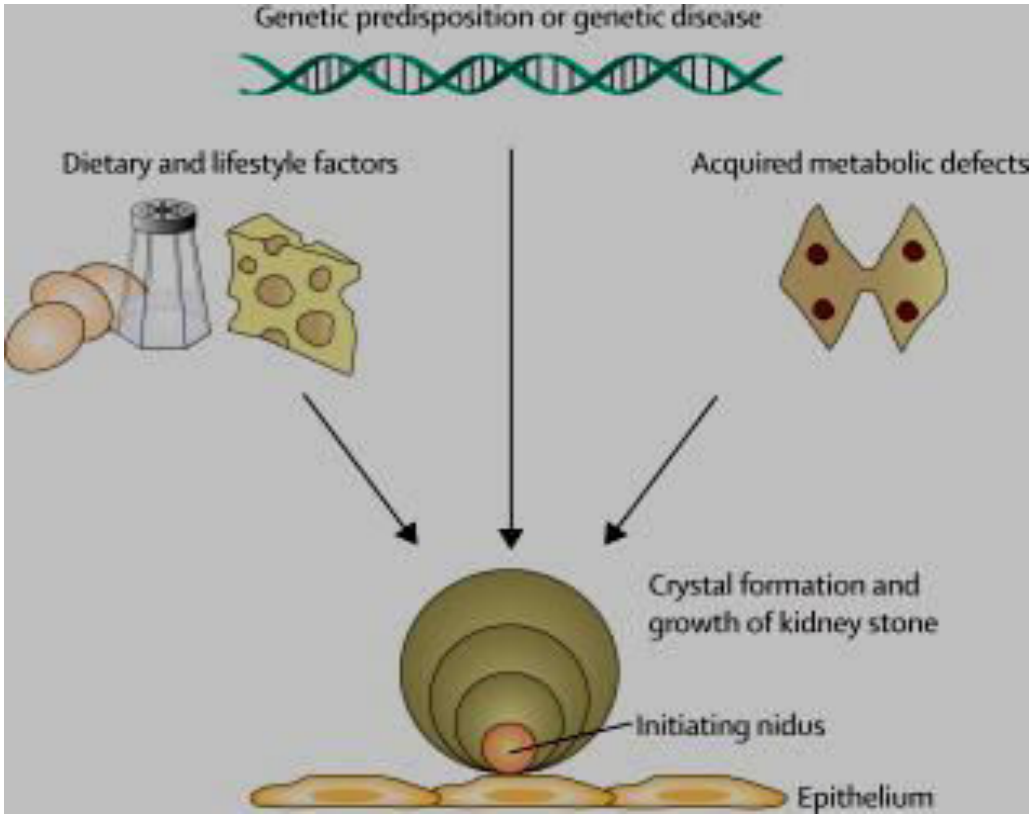


Figure No: 4 Pathogenesis of kidney stones

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.

URINARY SUPERSATURATION AND CRYSTALLIZATION:

Urinary supersurturation is the driving force behind crystal formation in the kidneys. Since formation of crystalline particles must obviously start from supersaturation, 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 normally excrete millions of urinary crystals daily, indicating at least transient development of supersaturation. 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 activity and the relative supersaturation of calcium oxalate processes are largely dependent on solution composition. A variety of urinary constituents may affect solution supersaturation because of their activity as chelaters. For instance, by forming soluble complexes with calcium and oxalate, respectively, citrate and magnesium reduce free ion.

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 stone 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.

CRYSTAL GROWTH:

Once a crystal nucleus has achieved a critical size and relative supersaturation 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 functions. 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, a1-acid glycoprotein, a1-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 2-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 causes only 9.9% inhibition of Calcium oxalate crystal growth.

CRYSTAL AGGREGATION:

The process whereby crystals in solution stick together to form larger particles is called aggregation. Some researchers have proposed that crystal aggregation is the most important step in stone formation. Although crystal growth is definitely a step in 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 in 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 .

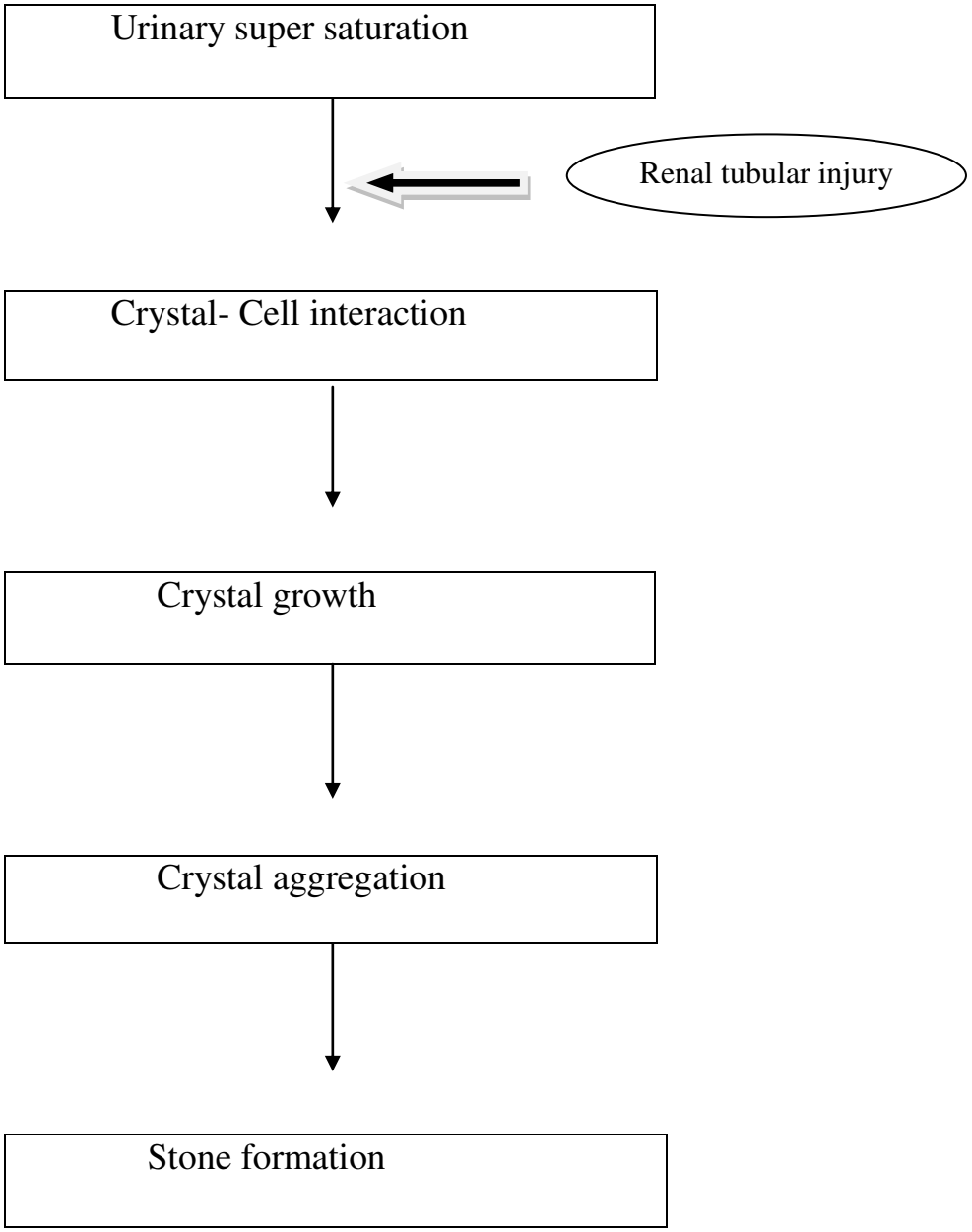
CRYSTAL CELL INTERACTION:

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 supersaturation.

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 what is generally meant by crystal–cell interactions. 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 calcium oxalate monohydrate 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 calcium oxalate monohydrate 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 calcium oxalate monohydrate 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 interaction is an essential element in the development of urinary stone disease. 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. [17]


Urinary super saturation
Renal tubular injury
Crystal- Cell interaction
Crystal growth
Crystal aggregation
Stone formation

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.

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 aetiological 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 crystallisation.

Pyrophosphates:

At low concentrations, 16 mM, pyrophosphate inhibits calcium oxalate monohydrate crystal growth by 50%. The urinary pyrophosphate level is in the range of 20–40 mM and therefore, theoretically levels are high enough to inhibit Calcium oxalate and Calcium phosphate crystallisation. 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 and this action probably mediated by formation of 1.25 (OH)2 – vitamin D. Sharma et al reported low 24-hour urinary excretion of pyrophosphate in stone formers (50.672.16 mmol/24 h) as compared to normal subjects (71.465.46 mmol/ 24 h) ($p < 0.01$) . 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.

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 Super Saturation. 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.

Inter-alpha-trypsin inhibitor family of proteins:

Inter-a-inhibitor (IaI) belongs to the Kunitz-type protein superfamily, a group of proteins possessing a common structural element (kunin) and the ability to inhibit serine proteases .I aI 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 Kunitz type protease inhibitor found in human amniotic fluid and urine, exhibits anti-inflammatory and anti-metastatic functions in animals and humans . It is expressed mainly in the proximal tubules and the 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 IaI and Calcium oxalate stone formation.

Osteopontin (Uropontin):

Osteopontin (OPN) is a negatively-charged aspartic acid rich protein that inhibits growth of Calcium oxalate crystals in a supersaturated solution. OPN is intimately involved in the regulation of both physiological and pathological mineralization. OPN is a phosphorylated protein of wide tissue distribution that is found in association with dystrophic

calcification including in the organic matrix of kidney stones. OPN is synthesised within the kidney and present in the human urine at levels in excess of 100 nM.

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 inviter.

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 saliyated glycoforms of UPTF1 afford protection against Calcium oxalate stone formation, possibly by coating the surface of Calcium oxalate crystals.

Tamm-Horsfall Protein:

Tammand 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'sloop (TAL) with exception the 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 h in humans.

THP may be involved in the pathogenesis of cast nephropathy, urolithiasis, and tubule interstitial 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 calcium oxalate monohydrate 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.

Glycosaminnoglycans:

Glycosaminnoglycans (GAGs) have been identified as one of the macromolecules present in the stone matrix. Chondroitin sulphate, heparin sulphate and hyaluronic acid are excreted in the urine. Recently, the main GAGs found in stone matrix were identified as heparan 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 Super Saturation. 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.

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 (CaCO3) in renal stones suggested that crystals of CaCO3 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.

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 Super Saturation required to initiate crystallisation and therefore promote Calcium oxalate crystallisation. Strong geometric similarities between the crystals of uric acid dehydrate and calcium oxalate monohydrate may promote over growth of one on the other, a process similar to the relationship between apatite and calcium oxalate monohydrate .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 factors that modulate these crystal-cell interactions could stimulate the initiation of an intrarenal stone. [18]

DIAGNOSIS:

Diagnosis of kidney stones is made on the basis of information obtained from the history, physical examination, urinalysis, and radiographic studies. Clinical diagnosis is usually made on the basis of the location and severity of the pain, which is typically colicky in nature (comes and goes in spasmodic waves). Pain in the back occurs when calculi produce an obstruction in the kidney. Physical examination may reveal fever and tenderness at the costovertebral angle on the affected side.

Imaging studies:

In people with a history of stones, those who are less than 50 years of age and are presenting with the symptoms of stones without any concerning signs do not require helical CT scan imaging. A CT scan is also not typically recommended in children.

Otherwise a noncontrast helical CT scan with 5 millimeters (0.2 in) sections is the diagnostic modality of choice in the radiographic evaluation of suspected nephrolithiasis. All stones are detectable on CT scans except very rare stones composed of certain drug residues in the urine, such as from indinavir. Calcium-containing stones are relatively radiodense, and they can often be detected by a traditional radiograph of the abdomen that includes the kidneys, ureters, and bladder (KUB film). Some 60% of all renal stones are radiopaque. In general, calcium phosphate stones have the greatest density, followed by calcium oxalate and magnesium ammonium phosphate stones. Cystine calculi are only faintly radiodense, while uric acid stones are usually entirely radiolucent.

Where a CT scan is unavailable, an intravenous pyelogram may be performed to help confirm the diagnosis of urolithiasis. This involves intravenous injection of a contrast agent followed by a KUB film. Uroliths present in the kidneys, ureters, or bladder may be better defined by the use of this contrast agent. Stones can also be detected by a retrograde pyelogram, where a similar contrast agent is injected directly into the distal ostium of the ureter (where the ureter terminates as it enters the bladder).

Renal ultrasonography can sometimes be useful, because it gives details about the presence of hydrone phrosis, suggesting that the stone is blocking the outflow of urine. Radiolucent stones, which do not appear on KUB, may show up on ultrasound imaging studies. Other advantages of renal ultrasonography include its low cost and absence of radiation exposure. Ultrasound imaging is useful for detecting stones in situations where X-rays or CT scans are discouraged, such as in children or pregnant women. Despite these advantages, renal ultrasonography in 2009 was not considered a substitute for noncontrast

helical CT scan in the initial diagnostic evaluation of urolithiasis. The main reason for this is that, compared with CT, renal ultrasonography more often fails to detect small stones (especially ureteral stones) and other serious disorders that could be causing the symptoms.

Laboratory examination:

Laboratory investigations typically carried out include:

- microscopic examination of the urine, which may show red blood cells, bacteria, leukocytes, urinary casts, and crystals;
- urine culture to identify any infecting organisms present in the urinary tract and sensitivity to determine the susceptibility of these organisms to specific antibiotics;
- complete blood count, looking for neutrophilia (increased neutrophil granulocyte count) suggestive of bacterial infection, as seen in the setting of struvites stones .
- renal function tests to look for abnormally high blood calcium blood levels (hypercalcemia);
- 24 hour urine collection to measure total daily urinary volume, magnesium, sodium, uric acid, calcium, citrate, oxalate, and phosphate;
- collection of stones (by urinating through a Stone Screen kidney stone collection cup or a simple tea strainer) is useful. Chemical analysis of collected stones can establish their composition,

which in turn can help to guide future preventive and therapeutic management.[23]

Current management and treatment of Urolithiasis is aimed in the prevention and treatment of recurrent urolithiasis is to increase the daily fluid intake to at least 2.5 L to 3 L per day along with pain controlling drugs and medications to monitor salts that may increase or reduce formation of stones. On the contrary, most stones with a diameter >8 mm will ultimately necessitate intervention. Many allopathic agents like thiazide diuretics (hydrochlorothiaide), alkali (potassium citrate), allopurinol, sodium cellulose phosphate (SCP), penicillamine (cuprimine), analgesic (diclophenac sodium), bisphosphonates, potassium phosphate and probiotics (Oxalobacter formigenes) are used in treating stones. Thiazide diuretics (e.g., hydrochlorothiazide, chlorthalidone and indapamide) produce an increase in tubular reabsorption of calcium, which diminishes calciuria, and hence are effective in reducing calciuria and stone recurrence. However, most of these standard pharmaceutical drugs used to prevent and cure urolithiasis are not effective in all cases,

costly, quite common reoccurrences, risks of long term fertility, potential side effects and no guarantee. [1]

A large number of Indian medicinal plants have been used in the treatment of urolithiasis and they have been reported to be effective with fewer side effects.

Before the advent of lithotripsy and ureteroscopy, most patients with symptomatic upper tract calculi underwent open surgical lithotomy. However, lithotripsy and ureteroscopic extraction have dramatically reduced the role of open stone surgery. Despite these advancements, techniques such as extracorporeal shock wave lithotripsy and percutaneous nephrostolithotomy do not assure the prevention of recurrence of the stone. They cause side effect such as haemorrhage, hypertension, tubular necrosis, and subsequent fibrosis of the kidney leading to cell injury, and ultimately recurrence of renal stone formation. Also these methods are costly, non-affordable by the poor section and the re-occurrence rate is also high (50-80%). Thus, even with the improved understanding of the mechanisms of stone formation and treatment, the worldwide incidence of urolithiasis is quite high and there is no truly satisfactory drug for treatment of renal calculi. [1],[20]

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 used only rarely since the introduction of ESWL, which has revolutionized 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 may cause acute renal injury, a decrease in renal function and an increase in stone recurrence. Furthermore, although some drugs used to prevent the disease have some positive effects, they are not effective in all patients and often have adverse effects that compromise their use in long-term medical 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. [8]

The worldwide incidence of urolithiasis is quite high and there is no truly satisfactory drug for treatment of renal calculi. A large number of Indian medicinal plants have been used in the treatment of urolithiasis and they have been reported to be effective with fewer side effects. [1]

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 assumed greater importance. [9]

2. REVIEW OF LITERATURE

2.1 Literature review on anti urolithiatic studies:

Sathish R et al., (2018) studied the in vitro anti-lithiatic effect of *Ipomoea batatas* (*Convolvulaceae*) leaves and tuberous roots. The obtained ethanolic extract of *I. batatas* leaves and tuberous roots (EIBL and EIBR) and aqueous extract of *I. batatas* leaves and roots (AIBL and AIBR) were used for this in vitro study. The dissolution method of calcium oxalate by titrimetry method and calcium phosphate by colorimetric method was studied. Nucleation and aggregation of calcium oxalate crystals were determined by a spectrophotometric assay. Results indicated the estimation of calcium oxalate by titrimetry method, the *I. Batatas* leaves and roots have very significant ($p<0.01$) capability to dissolve calcium oxalate. Percentage dissolution of calcium oxalate crystals was found to be 37.53%, 22.74%, 39.74%, and 24.28% for EIBL, AIBL, EIBR, and AIBR, respectively. In the estimation of calcium phosphate by colorimetric method, the percentage dissolution of calcium phosphate crystals by EIBL, AIBL, EIBR, and AIBR was found to be 67.15%, 43.17%, 76.74%, and 47.96%, respectively. The *I. batatas* leaves and roots were significantly ($p<0.01$) dissolved calcium phosphate also. The results were clearly shown that *I. batatas* extracts significantly ($p<0.01$) inhibited both nucleation and aggregation of calcium oxalate crystals by concentration-dependent manner. The maximum percent inhibition of calcium oxalate nucleation by EIBL, AIBL, EIBR, and AIBR was found to be 59.09%, 50.0%, 84.09%, and 47.73%, respectively, at 1000 µg/ml.[25]

Abdullah H et al., (2017) studied anti-urolithiatic of the hydroalcoholic extracts of *Phoenix dactylifera Linn* seeds (roasted and non-roasted) in calcium oxalate urolithiasis in male albino rats. Lithiasis was induced by oral administration of ammonium chloride 1% and ethylene glycol (0.75 %v/v) in male albino rats for 28 days. The non-roasted and roasted extracts (500 mg/kg) were administered orally from the 15th day as a curative regimen urine analysis, serum analysis, biochemical analysis of kidney homogenate and histopathological study were performed. Results indicated the urine volume, urine magnesium and kidney GSH levels were decreased as well as calcium excretion in urine, serum creatinine and urea, kidney MDA and NO contents were increased in lithiatic group as compared to control group. Treatment with both hydroalcoholic seed extracts restored urine volume, magnesium and kidney GSH,

MDA and NO levels while treatment with of non-roasted extracts reduced the elevated level of urinary calcium, serum creatinine and urea levels as compared to lithiatic group. Histopathological examination revealed tubular degeneration, dilatation, presence of CaOx crystals in the lumen of renal tubules and intense interstitial mononuclear cell infiltration in the lithiatic control group. These histopathological alterations were markedly regressed in other treated groups. The results indicated that the hydroalcoholic extracts of *Phoenix dactylifera Linn* seeds either in non-roasted or roasted state have potent antiurolithiatic activity against calcium oxalate urolithiasis induced by ethylene glycol in male albino rats.[26]

Chandra Shekhar Tailor et al ., (2015) studied the, ethanolic & aqueous extracts of roots and rhizomes of *Hedychium coronarium J. Koenig* plant were evaluated for their potential to dissolve experimentally prepared kidney stones like calcium oxalate by titrimetic method with an invitro model and Antioxidant activity performed by DPPH scavenging assay method. Phytoconstituents were also isolated by chromatographic techniques of this plant species. For performing invitro antilithiatic activity titrimetic method was adopted and for antioxidant activity 1, 1-diphenyl-2-picryl hydrazyl (DPPH) scavenging assay method was adopted. Phytoconstituents were isolated by column and thin layer chromatographic techniques. Results indicated the ethanolic roots & rhizomes extract of this plant produced highest dissolution of stones when compare to standard drug cystone and at10 mg. concentration. Also this study showed alcoholic extract of roots & rhizomes of *Hedychium coronarium J. Koenig* plant in higher concentration possess best antioxidant potential as compare to standard ascorbic acid with IC50 value 9.0 and 18.9 µg/ml. for ascorbic acid and alcoholic extract respectively. Isolated phytoconstituent from alcoholic extract of this plant was 8a, hydroxy hedychilactone and its structure was confirmed by IR, NMR and Mass spectroscopic datas. [27]

Kamil M et al ., (2015) evaluated the effect of plant formulation (*Ziziphus spinachristi* leaves, *Alhagi maurorum* roots, *Zea mays* silk, *Hordeum vulgare* grains and *Pimpinella anisum* seeds) extract on kidney of Sodium oxalate induced urolithiasis in rats. The aqueous extract of plant formulation /standard drug cystone were administrated simultaneously at a dose of 100, 200 or 400 mg/kg body weight/day, along with sodium oxalate (70 mg/kg body weight/day) w/v) for 30 days. Significant changes were observed in body weight and kidney weight of sodium oxalate treated rats. Histopathological results showed disrupted renal parenchyma showing loss of structural arrangement of renal tubules; early degenerative changes in glomeruli and focal calcification in glomerulo-tubular structures were observed

in the renal tissue of urolithiatic rats .Cystic dilation of renal tubules with completing distraction of other due to necrosis with thickening of Bowman's capsules. Hydropic degeneration of proximal and distal renal tubules leading narrowing its Lumenic in sodium oxalate treated animals. Administration of plant formula extract or cystone along with sodium oxalate showed significant protective effect in body weight, kidney weight, with few stray areas of calcification in glomeruli and normal tubular structures, increased cellularity between tubules is clearly visible. Moreover, plant formula extract shows higher renoprotective than cystone. In conclusion, aqueous extract of the formulation of these plants has proved to be an effective drug in prevention of nephrolithiasis. [28]

R Saranya et al ., (2014) studied the inhibitory potential of *Beta vulgaris L.* leaf and root aqueous extracts against calcium oxalate crystallization under in vitro condition. Under in vitro condition, kidney stone formation was studied using three assays such as nucleation, aggregation and growth. Nucleation was studied by adding calcium chloride and sodium oxalate solution in the presence (0.1 to 9 mg/ml) and absence of aqueous extracts at 37˚C. For aggregation and growth calcium oxalate monohydrate crystals were prepared and studied. The effect of extracts on the formation and inhibition of stone forming stages were observed spectrophotometrically and analyzed through phase contrast microscope at 40X magnification. The result obtained showed that aqueous extracts of the leaves and root of *Beta vulgaris L.* have higher capacity to inhibit the crystal nucleation, aggregation and growth. When compared with leaf aqueous extract, root aqueous extract of beet root showed better inhibitory activity. Extracts inhibited the crystallization in solution; less and smaller particles were observed in the presence of extracts. These results were further confirmed with the aggregation and growth assay, the extracts prevented the aggregation and growth of formed CaOx particles and it kept the crystals as dispersed. From this in vitro study we can conclude that beet root extract has excellent therapeutic potential as diuretic and promoting the inhibition of the formation of CaOx crystals. Also the phytocompounds present in this plant may be responsible for the anticrystallization activity. [29]

Kumkum Agarwal et al., (2014) evaluated the *Ocimum gratissimum L.* has been used to treat various diseases including urinary stone diseases, since ancient time in India. The inhibition of in-vitro calcium-oxalate crystal formation by *Ocimum gratissimum L.* extract was investigated by different methods i.e. nucleation assay and synthetic urine assay. In nucleation assay, the aim was to evaluate the effectiveness of different concentrations of the extract (100-1000 mg/ml) on calcium oxalate crystallization in-vitro while in synthetic urine

method the percentage inhibition and growth of the calcium oxalate monohydrate crystals from synthetic urine at different % concentrations of extract (25-100%) was investigated. In both the assay % inhibition for calcium oxalate crystal formation was found directly proportional to the increase in concentration of the plant extract with maximum inhibition of 66.08% at 1000 mg/ml, while in synthetic urine assay maximum inhibition was 62.07 % at 100% concentration of extract. Thus *Ocimum gratissimum L.* was found to be a potent and promising antiurolithiatic agent, which is in accordance with its use in traditional medicine.[30]

R Naga kishore et al., (2014) evaluated the effects of some traditional medicinal plants i.e., *Brassica oleracea Gongylodes* and *Desmostachya bipinnata* in combination and in alone on experimentally-induced (urolithiasis) kidney stones. Urolithiasis in animals was induced experimentally by administration of ethylene glycol (0.75% v/v) with ammonium chloride (1% w/v) in drinking water for ten days. The aqueous extract of both plants were administered alone and in combination to urolithiasis induced test group rats at a dose of 400 mg/kg respectively for 10 days. After 10 days, renal function parameters measured such as increase in the urine urea, uric acid, and calcium and creatinine levels, which reflect the deposition of calcium oxalate in the kidneys. Results indicated the serum analysis showed significant increase in the serum uric acid, serum creatinine, blood urea and calcium in urolithiasis control group rats. In addition, vehicle treated induction control group rats showed significant increase in the biochemical parameters. Daily oral treatment with all most all extracts not significantly decreased the quantity of calcium oxalate deposited in the kidneys but also reverted all the biochemical changes induced by calcium oxalate urolithiasis.[31]

Narendra vyas et al., (2013) evaluated the antiurolithiatic activity of ethanolic extract of roots (ELC 200 mg/kg) and oleanolic acid (OA 60mg/kg, O.A. 80mg /kg, O.A. 100mg/kg) isolated from roots of *Lantana camara* in albino wistar male rats using zinc disc implantation induced urolithiatic model. The group in which only zinc disc was implanted without any treatment showed increase in calcium output (23 ± 2.7mg/dL). Cystone receiving animals showed significant protection from such change ($P < 0.01$). Treatment with OA and ELC significantly reduced the calcium output at a dose of OA 60mg/kg ($P < 0.01$), OA80mg/kg ($P < 0.01$), ELC 200mg/kg ($P < 0.01$), and OA 100mg/kg ($P < 0.001$), as compared with zinc disc implanted group. The average weight of zinc discs along with the deposited crystals in the only disc implanted group was found to be 111 ± 8.6mg. Group that received Cystone

500mg/kg showed significant reduction in the depositions ($P < 0.001$). Similarly, the rats which received OA and ELC showed reduced formation of depositions around the zinc disc ($P < 0.001$). The X-ray images of rats also showed significant effect of OA and ELC on urolitiasis. Thus, OA and ELC showed promising antiurolithiatic activity in dose dependant manner. [32]

Ajij Ahmed et al., (2013) investigated the antiurolithiatic effect of the hydro alcoholic extract of *Adiantum capillus veneris Linn* in male Sprague Dawley rats. The effects of oral administration of hydro alcoholic extract of test drug were studied on calcium oxalate urolithiasis. A total of 48 rats were used for the study. The animals were divided into six groups of eight animals each. Plain control rats were treated with distilled water only, throughout the study period, whereas in other groups nephrolithiasis was induced by providing drinking water containing 0.75% ethylene glycol and 1% ammonium chloride for 7 days. Thereafter, urine was examined for the presence of crystals. Negative control group A rats were sacrificed after 7 days, whereas negative control group B was left untreated up to the end of study. Test groups were treated with 127.6 mg/kg and 255.2 mg/kg of test drug and standard control with Cystone (750 mg/kg) for 21 days. At the end of experiment, number of crystals in urine and levels of calcium, phosphorus, urea and creatinine in serum were observed. Histopathological study of the kidney was done by light microscopy. Results indicated the Urine microscopy showed significant reduction (po0.001 and po0.01) in the number of crystals in test groups A and B respectively. Serum levels of calcium, phosphorous, and blood urea were found to be decreased significantly in all the groups. In both the test groups, serum creatinine level was found to be similar as in plain control. The animals treated with test drug showed much improvement in body weight. Histopathology of kidney showed almost normal kidney architecture in treated groups. The above findings indicated the anti urolithic activity of *Adiantum capillus veneris Linn*, and thus, validate the claims of Unani physicians for its medicinal use in urolithiasis. [33]

Nirmala Devi R et al. (2013) studied the alternative treatment by using *Aerva lanata*. The effect of aqueous extract of dried flower of *Area lanata* against ethylene glycol. Induced renal calculi in albino wistar rats and cystone tablets in popular medicine for kidney stone patients. This two extracts comparative study, a renal calculus was induced in rats by ingesting 0.75% ethylene glycol in drinking water for 28 days and was concentrated on estimation of calcium, oxalate, phosphate, magnesium excretion in the urine. This elevated urinary calcium with high urinary oxalate might lead to calcium oxalate stone formation;

following administration of extracts significantly lower urinary calcium and oxalate were observed to compare with the cystone. This enabled us to conclude that the extract is antilithiatic activity. [34]

S Kamesh waran et al., (2013) studied the *Tecoma stans* flowers have been preferred for their antiurolithiatic activity on experimentally induced urolithiatic rats. Antiurolithiatic activity of aqueous and methanolic extracts of *Tecoma stans* (AETS & METS) was carried out on ethylene glycol (0.75% v/v) induced urolithiasis in rats. Treatment with aqueous extract (200mg/kg, p.o) and methanolic extract (250mg/kg, p.o) of *T.stans* flowers significantly lowered ($P<0.001$) the increased levels of oxalate, calcium and phosphate in urine and also significantly reduced ($P<0.001$) their retention in kidney. The treatment with Aqueous extract and Alcoholic extract of *Tecoma stans* flowers significantly ($P<0.001$) lowered the elevated serum levels of Blood urea nitrogen, creatinine and uric acid in both regimens. The histopathological study of the kidney also supported the above results. The results were comparable to that of standard drug (Cystone). The presented data indicate that administration of AETS and METS to rats with experimentally-induced urolithiasis reduced and also prevented the formation of urinary stones, supporting folk information regarding antiurolithiatic activity of the plant. The reduction in the stone forming constituents in urine and renal tissue brought about by *T.stans* could contribute to its antiurolithiatic property. [35]

D G Baheti et al., (2013) studied the effects of a polyherbal formulation (PHF) on experimentally-induced kidney stones. Oxalate urolithiasis in male rats was induced experimentally by administration of 0.75% v/v ethylene glycol with 1% w/v ammonium chloride in drinking water for three days followed by only 0.75% v/v ethylene glycol for 25 days. The PHF was administered to urolithiasis induced test group rats at three doses i.e. 200, 300 and 400mg/kg respectively for 28 days. After 28 days, highly significant deposition of calcium oxalate in the kidneys was noticed along with increase in the urine volume, urinary oxalate, calcium, levels and magnesium levels in urolithiasis control group rats as compared to normal group rats. The serum analysis showed significant increase in the serum uric acid, serum creatinine, and blood urea in urolithiasis control group rats. In addition, vehicle treated induction control group rats showed significant increase in the biochemical parameters such as ACP, ALP, AST, ALT levels and decrease in LDH levels in the kidney homogenate which indicated the induction of urolithiasis. Daily oral treatment with PHF at doses 300 and 400mg/kg significantly decreased the quantity of calcium oxalate deposited in the kidneys but also reverted all the biochemical changes induced by calcium oxalate urolithiasis thus

supporting its traditional claim. 200 mg/kg dose of PHF however, was found to be insignificant in these regards. [36]

NR Kachchhi et al., (2012) evaluated the antiurolithiatic activity of methanolic extract of *Celosia argentea* roots (CaME) in male albino wistar rats. Ethylene glycol (0.75% v/v in drinking water; 28 days) induced urolithiasis preventive model was used to study the effect of CaME low dose (250 mg/kg; p.o.) and high dose (500 mg/kg; p.o.). Cystone (750 mg/kg; p.o.) was used as a standard. At the end of the treatment changes in various physical parameters, promoters, inhibitors, renal function markers in urine and serum samples and antioxidant parameters and histopathology of kidneys were observed. Treatment groups significantly prevented improvement in urinary pH, diuresis and body weight. All the treatments significantly prevented the rise in promoters like calcium, oxalate, uric acid, and inorganic phosphate and increased the levels of magnesium and citrate like inhibitors in various biological samples. Renal function impairment and oxidative stress was also prevented by the treatment as observed by BUN and creatinine analysis and analysis of MDA, proteins, catalase and histopathology respectively. Thus methanolic extract of *Celosia argentea* roots has proved to be an effective drug in prevention of urolithiasis. [37]

Surendra K. Paretato et al., (2011) studied the inhibitor effect of hydroalcoholic extract of *Achyranthes indica Linn*. On the crystallization of calcium oxalate in synthetic urine. Our study of the calcium oxalate crystallization is based on change in turbidity followed at 620nm by means of a spectrophotometer. The calcium oxalate formation is induced by the addition of 0.01M sodium oxalate solutions in synthetic urine. The addition of inhibitor with various concentrations (10%, 20%, and 40%) enabled us to give information on the percentage of inhibition. The comparison between the turbidimetric slopes with and without inhibitor gives the effectiveness of inhibitor for the hydroalcoholic extract. By comparing the photomicrographs of with and without inhibitor, we concluded that the extract of *Achyranthes indica Linn*. acts on nucleation as well as crystal growth stage. The hydroalcoholic extract of the *Achyranthes indica Linn*. remarkably inhibits of crystal formation in calcium oxalate urinary lithiasis. [38]

Manabu T moriyama et al., (2009) evaluated the mechanism of Urocalun, an extract of *Quercus salicina Blume / Quercus stenophylla Makino* (QS), in the treatment of urolithiasis. Rat calcium oxalate urolithiasis was induced by oral administration of ethylene glycol and the vitamin D3 analog alfa-calcidol for 14 days. QS Extract was repeatedly given to rats. After

the last administration, biochemistries in urine and plasma, renal calcium, and urinary malondialdehyde (an oxidative stress marker) were measured. Results indicated the Ethylene glycol and alfa-calcidol treatment increased urinary malondialdehyde and renal calcium levels. This increase was significantly suppressed by the administration of QS extract, suggesting that the inhibition of renal calcium accumulation by QS extract is due to its antioxidative activity. Conclusions: These findings suggest that the antioxidative activity of QS extract plays a role in the prevention of stone formation and recurrence in urolithiasis. [39]

2.2 Literature review on various pharmacological activities of *Phaseolus vulgaris Linn*

Louise Anin, Anin Atchibri et al., (2017) evaluated the hypoglycemic and lipid-lowering effect of the aqueous extract of *Phaseolus vulgaris* in rat rendered diabetic by diet. The effects of the nutritional supplementation of *Phaseolus vulgaris*, at the rates of 300 mg / Kg in high carbohydrate and high fat diets, diabetic Wistar rat race, for 6 weeks, were analyzed on several measures of glucose and lipid metabolism. The aqueous extract of bean seed germinated is highly effective in reducing the level of glucose in the blood of diabetic rats, while increasing insulin levels. The concurrent negative biochemical factors in the type 2 diabetes, such as triglycerides, total cholesterol, and LDL cholesterol undergo a decrease, while the HDL cholesterol increased in diabetic rats having ingested the aqueous extract of bean seed. Thus, *Phaseolus vulgaris* is an important contribution in the formulation of dietetic diets, because it constitutes a useful supplement which would permit to decrease the dosages of oral hypoglycemic in the case of the non insulino-dependent diabetes, by giving a greater comfort of life to the patient, and by decreasing the importance of the side effects related to the anti-diabetic medications which are far from being negligible. [40]

Salman Ahmed et al., (2016) studied all possible morphological features of calcium oxalate monohydrate and calcium oxalate dihydrate crystals and their habits in case of inhibition. In this the study was carried out on glass slide to observe the growth and inhibition of calcium oxalate monohydrate (COM) crystals by using infusions (5-20%) of *Macrotyloma uniflorum (Lam.) Verdc, Phaseolus lunatus Linn. and Phaseolus vulgaris Linn*. The reagent of double diffusion gel technique was used for this purpose. Results indicated that the Calcium oxalate crystals are divided into three types, calcium oxalate monohydrate, calcium oxalate dihydrate and calcium oxalate trihydrate. These types are further divided into sub types on the basis of their morphology. In case of calcium oxalate monohydrate (COM), these crystals are donut, dumbbell, needles, platy, prismatic, rosette, round edges and X-shaped. Whereas, calcium

oxalate dihydrate (COD) are reported as the elongated large rods and tetragonal bipyramidal forms. In the present study dendritic or arborescent (tree like platy crystals) were observed for the first time as the part of a COM growth. Long chain loose agglomerates and compact aggregated crystals are the common pattern of calcium oxalate crystals. All tested infusions caused growth inhibition of calcium oxalate crystals. Smaller zones of nucleation and defected shape of the grown crystals; declare as different patterns of growth inhibition. Conclusion: This study gives an extensive information about morphology, aggregation and growth inhibition of calcium oxalate crystals. [41]

Mariana Yuriivna Kyznetsova et al ., (2015) studied the social significance of diabetes mellitus lies in the fact that in addition to significant prevalence, this disease is associated with many complications. To facilitate the course of diabetes and its complications medicinal plants are widely used in traditional medicine. One of such plants is kidney bean (*Phaseolus vulgaris*). This plant is used in traditional medicine, especially for the secondary complications of diabetes. Since complications of diabetes are often associated with increased oxidative stress, the study of antioxidant properties of *P. vulgaris* is important to clarify the mechanism of its therapeutic effect. Present investigation shows that long-term oral administration of aqueous *P. vulgaris* pods extract in dose of 200mg/kg b.w. besides its pronounced hypoglycemic action also has a positive influence on the liver and kidney function markers in STZ-treated diabetic rats. The extract also inhibits free radical production and lipid peroxidation and activates antioxidant enzymes in liver and kidneys of rats with STZ-induced diabetes. Thus, our data reveal antioxidant properties of aqueous *P. vulgaris* pods extract that might have beneficial effect in treatment of diabetes. [42]

M. R. Pradeepkumar et al ., (2015) studied an objective of developing some novel analgesic and anti-inflammatory natural agents with fast acting and low toxicity profile herein, the different extracts of *Phaseolus vulgaris (Linn)* seeds were evaluated for analgesic and anti-inflammatory activities using glacial acetic acid induced writhing and carrageenan induced rat paw oedema method respectively. For screening of the extracts for analgesic and anti-inflammatory activities aspirin and diclofenac were used as standard drugs respectively. Petroleum ether extract exhibited significant analgesic and anti-inflammatory activities. The petroleum ether extract can be considered as a potential candidate for analgesic and anti-inflammatory activities. The presence of steroids and flavonoids in petroleum ether extract of *Phaseolus vulgaris Linn*. seeds could be attributed for the analgesic and anti-inflammatory activities. [43]

N. Sree Lakshmi et al., (2015) evaluated antiurolithiatic activity of ethanolic seed extract of *Phaseolus vulgaris* (EPV) on in vitro calcium oxalate (CaOx) crystallization. In vitro CaOx crystallization was assessed by employing crystal nucleation, aggregation and growth assay methods in the presence and absence of EPV at the concentration range of 100-1000 µg/ml. Cystone (100-1000 µg/ml) was used as positive control. Effect of EPV on the rate of crystal nucleation, aggregation and growth were measured spectrophotometrically and their percentage inhibition was calculated. Morphological characteristics of the crystals in control, cystone and EPV were observed under phase contrast microscope. EPV and cystone produced dose-dependent inhibition of crystal nucleation, aggregation and growth. IC50 values of cystone on nucleation, aggregation and growth were found to be 166.40±22.31, 343.69±27.51 and 360.10±17.52 µg/ml respectively, whereas with EPV, IC50 values were 489.10±49.27, 580.11±53.16 and 725.70±29.33 µg/ml respectively. Results of the present in vitro study suggest that ethanolic seed extract of *P. vulgaris* possess calcium oxalate crystal inhibition activity on crystal nucleation, aggregation and growth suggesting its antiurolithiatic activity. [44]

Luka CD et al., (2013) studied the aqueous extract of *Phaseolus Vulgaris L.* (Red Kidney Beans) was investigated for its effects in alloxan induced-diabetic rats. Twenty four albino rats were randomly allocated into four groups (A-D) of six rats each such that group A (diabetes control) received 0.5 mL distilled water, group B (diabetes) received 400 mg/kg bwt of extract, group C (normal control) received 0.5 mL of distilled water while group D (normal) received 400 mg/kg bwt of extract, all extract were orally administered once daily for 14 days. Diabetes was induced in groups A&B by single interperitonial injection of 150 mg/kg alloxan monohydrate. Phytochemical screening indicated the presence of alkaloids, balsam, flavonoids, saponins, tannins, cyanogenic glycosides, terpenes and steroids. The hypoglyceamic potential of *Phasoelus vulgaris L.* was expressed in diabetes treated rats. Blood glucose, total protein, albumin and cholesterol levels of the diabetes treated rats and normal treated rats were not significantly ($p>0.05$) altered when compared with the control rats. However, these values were significantly ($p<0.05$) increased in diabetes untreated rats. Aqueous extracts of *Phaseolus vulgaris L.* at 400 mg/kg body weight also significantly reduce ($p<0.05$) the values of ALT, AST and ALP when compared to high values of the enzymes observed in diabetes untreated rats. Extract had no significant ($p>0.05$) effects on PCV and Hb in all groups when compared to the normal control. The study showed that the aqueous extract of *Phasoelus vulgaris L.* leaves possess hypoglycaemic, antidiabetic

properties and ameliorating the high levels of marker enzymes observed in diabetes untreated rats. [45]

L. Ocho-Anin Atchibri et al (2010) studied the Seeds of *Phaseolus vulgaris* were given individually at different doses to different batches of rats (both normal and hyperglicemic rats) after an overnight fast. Seeds contain the bioactive components – alkaloids, flavonoids, fiber, proteins, tannins, terpenoids, saponins, quercetin, anthocyanin and catechin. The blood glucose levels were measured at 0, 1, 2, 3, 4, 5 and 6 h after the treatment. Most active doses were further studied to dose-dependent (300, 200 and 100 g/kg bw) antihyperglycemic effects alone and in combination with glibenclamide (0.20, 0.10 and 0.05 g/kg bw). Seeds of *P. vulgaris* at a dosage of 300 g/kg bw is showing maximal blood glucose lowering effect in diabetic rats after third hour. The antihyperglycemic activity of *P. vulgaris* seeds was compared with the treatment of glibenclamide, an oral hypoglycemic agent. The combination of seeds of most dose (300 mg/kg bw) and higher dose of glibenclamide (0.20 g/kg bw) showed safer and potent hypoglycemic as well as antihyperglycemic activities without creating severe hypoglycemia in normal rats. [46]

L. Pari et al (2003) studied the Oral administration of 200 mg/kg of aqueous extract of *Phaseolus vulgaris* pods (PPEt) to diabetic animals for 45 days resulted in a significant decrease in blood glucose, glycosylated haemoglobin and significant increase in total haemoglobin and plasma insulin. Similarly oral administration of PPEt to normal animals resulted in a significant hypoglycemic effect. The activities of hepatic hexokinase, glucose 6-phosphatase, fructose-1, 6-bisphosphatase and glucose-6-phosphate dehydrogenase, a lipogenic enzyme, were measured in the liver of normal and experimental animals. The activities of the lipogenic enzyme and hexokinase were significantly decreased, whereas the activities of gluconeogenic enzymes were significantly increased in the diabetic liver. Both PPEt and glibenclamide reversed the activities of these enzymes to near normal levels. PPEt was more effective than glibenclamide. The results indicate that the administration of PPEt to diabetic animals normalizes blood glucose and causes a marked improvement of altered carbohydrate metabolic enzymes during diabetes. [47]

L Hangen et al., (2002) investigated (*Phaseolus vulgaris*) an important food staple in many traditional diets. There is limited evidence to suggest an inverse relationship between bean consumption and colon cancer. The objective of this study was to determine whether consumption of black beans and/or navy beans would reduce colon carcinogenesis in rats.

Rats were fed a modified AIN-93G diet (control) or diets containing 75% black beans or 75% navy beans for 4 wk, and then colon cancer was initiated by administration of two injections of azoxy methane1 wk apart. At 31 wk after these injection, the incidence of colon adeno carcinomas was significantly lower ($P < 0.05$) in rats fed the black bean (9%) and navy bean (14%) diets than in rats fed the control diet (36%). Total tumor multiplicity was also significantly lower in rats fed the black bean (1.1) and navy bean (1.0) diets than in rats fed the control diet (2.2). The 44–75% reduction in colon carcinogenesis in rats fed beans was attributed to 1) more controlled appetites, leading to significantly less body fat, and 2) much greater concentrations of butyrate in the distal colon. It was concluded that eating black beans and navy beans significantly lowered colon cancer incidence and multiplicity. [48]

Subramanian Venkateswaran et al., (2002) studied the antioxidant effect of an aqueous extract of *Phaseolus vulgaris* pods, an indigenous plant used in Ayurvedic medicine in India, was studied in rats with streptozotocin-induced diabetes. Oral administration of *Phaseolus vulgaris* pod extract (PPEt; 200 mg/kg body weight) for 45 days resulted in a significant reduction in thiobarbituric acid reactive substances and hydroperoxides. The extract also causes a significant increase in reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in the liver and kidneys of rats with streptozotocin-induced diabetes. These results clearly show the antioxidant property of PPEt. The effect of PPEt at 200 mg/kg body weight was more effective than glibenclamide. [49]

SCOPE AND PLAN OF WORK

Scope:

Kidney stones are hard deposits of minerals like calcium either in urinary tract (urolithiasis) or in kidneys (nephrolithiasis). Along with plenty of fluid intake and urinary retention, the management of urolithiasis also includes combined surgical and medical approach using percutaneous nephrolithotomy (PCNL), extracorporeal shock wave lithotripsy (ESWL) and antibiotics. Surgical treatments are relatively costly, painful and require expert hand and availability of appropriate equipment. This has led to a trigger in search of natural resources possessing antiurolithiatic activity. [23] 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 rid of this painful disease.

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 rationale 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. Hence the search for antilithiatic drugs from natural sources has assumed greater importance. [24]

The literature search reveals the potential of several plant drugs such as seeds of *Trachyspermum ammi (L) Sprague* ex (umbelliferae), seeds of *Armoracia lopathifolia (*Brassicaceae*)*, rhizomes of *Bergenia ligulata* (Saxifragaceae), seeds of *Agrimonia eupatoria L*(fabaceae) in managing Lithiasis in animal models[50-52].

Phaseolus vulgaris, also known as kidney beans, is a common Indian dish. The seeds of *Phaseolus vulgaris* are gaining increasing attention as a functional or nutraceutical food, due to its rich variety of phytochemicals such as proteins, amino acids, complex carbohydrates, dietary fibers, oligosaccharides, phenols, saponins, flavonoids, alkaloids, and tannins, with potential health benefits. The seeds were claimed to possess diuretic activity and were commonly used in water retention treatment in pregnant women. Studies indicate that seeds of *Phaseolus vulgaris* were found to have activities such as enhancement of the bifidogenic, antioxidant, antimutagenic, anticarcinogenic, and antihyperglycemic effects. [58]

Phaseolus vulgaris Linn an easily available plant in India has been explored for the possession of analgesic and anti-inflammatory activity, Antioxidant activity, Anti-obesity, Antibacterial, Anticancer, Antidiabetic, Antifertility, Hepatoprotective, Hypolipidemic,

Trypsin and α-amylase inhibitor, Hyperglycaemic activity, Low nitrate stress, Anti tubular activity ,Atypical anti-psychotic, and Anti-fungal activity.[55-66]

The present study is aimed at evaluating the antiurolithiatic activity of the ethanolic extract and aqueous extracts of the seeds of *Phaseolus vulgaris* was investigated in Wistar rats against Calcium oxalate urolithiasis induced by ethylene glycol (EG) and ammonium chloride (AC) in drinking water.

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. Therefore the prophylactic use of the extracts in urolithiasis is also evaluated in the study.

OBJECTIVES:

1. To prepare aqueous and ethanolic extracts of *Phaseolus vulgaris* seeds and characterise its phytochemical nature by preliminary screening, TLC and quantification of some phyto constituents.
2. To evaluate the anti-oxidant action of *Phaseolus vulgaris* in 3 invitro models.
3. To investigate the anti urolithiatic activity of *Phaseolus vulgaris* in wistar albino rats by preventive and curative studies.

PLAN OF WORK

The present study is designed and carried out by the following methods to evaluate the anti urolithiatic activity of *Phaseolus vulgaris*

1. Collection and Authentication of plant.
2. Preparation of aqueous and ethanolic extracts of *Phaseolus vulgaris*
3. Identification of phytoconstituents of extracts.
    - Preliminary phyto chemical screening.
    - TLC study of extract.
4. Determination of Phyto constituents of extracts
    - Determination of Total Flavonoid content
    - Determination of Total Phenolic content
5. *In vitro* antioxidant studies on the extracts.
    - Determination of Nitric oxide Scavenging Assay.
    - Determination of Reducing Power Assay.
    - Determination of lipid Peroxidation Assay.
6. Pharmacological study:
    - Evaluation of anti lithiatic activity:

      A. Selection of animals

      B. Induction of Lithiasis

      C. Experimental design

      D. Assessment of antilithiatic activity.

      Urine analysis

      Serum analysis

      E. Histopathological Study of kidney.

4. PLANT PROFILE

Figure No: 5 *Phaseolus vulgaris Linn.* seeds

SCIENTIFIC CLASSIFICATION: [53]

Kingdom	:	Plantae
Division	:	Tracheophyta
Sub division	:	Spermatophytina
Class	:	Magnoliopsida
Order	:	Fabales
Family	:	Fabaceae
Genus	:	Phaseolus
Species	:	*vulgaris L.*

SYNONYMS OF PLANT: [54]

- *Phaseolus aborigineus Burkart*
- *Phaseolus communis Pritz.*
- *Phaseolus compressus DC.*
- *Phaseolus esculentus Salisb.*
- *Phaseolus nanus L.*

LOCAL NAMES OF PLANT: [55]

Burmese	:	Bo-Sa-Pe
Tamil	:	Naarila vithai avarai
French	:	Hericots verts
German	:	Gartenbohne
Gujarati	:	Phanasi
Hindi	:	Bakla
Kannada	:	Tingalavari
Marathi	:	Shravanghevda
Punjabi	:	Babri
Telugu	:	Bangalu

DISTRIBUTION: [56]

Tropical America. Beans are cultivated in all parts of the world and multitude of cultivars and beans type has been developed. The plant is indigenous to America.

DESCRIPTION: [57]

Bush varieties: Erect bushes 20–60 cm (8–20 in) tall, while pole or running varieties form vines 2–3 m (7–10 ft) long.

Leaves: Alternate, green or purple leaves, which are divided into three oval, smooth edged leaflets, each 6–15 cm (2–6 in) long and 3–11 cm (1–4 in) wide.

Flowers: The white, pink, or purple flowers are about 1 cm long

Pods: The pods are 8– 20 cm (3–8 in) long and 1–1.5 cm wide. These may be green, yellow, black, or purple in color, each containing 4–6 beans.

Seeds: The seeds are smooth, plump, kidney-shaped, up to 1.5 cm long, range widely in color, and are often mottled in two or more colors.

Fruit: The fruit are in lightly, blossomed, and shorter then their leaves

PHYTO CHEMICAL CONSTITUENTS: [58]

Anthocyanins, brassinosteroids, caffeic acid, catechic and coumestrol, daidzen, delphinidin, equol, ferulic acid, galactomanans, gallic acid, genistein, hemagglutinins, kaempferol, lectins, malvidin, myrecitin glycoside, para-coumaric acid, petunidin, phaseolamin, phaseolin, para-hydroxybenzoic acid, phytic acid, phytohaemagglutinin, proanthocyanidins, proanthocyanins, quercetin, robinin and vanillic acid. Allantoin, inositol, tyrosine, leucine, arginine, amino acid, L-pipecolic acid.

MEDICINAL USES: [59-66]

- Analgesic and anti inflammatory activity
- Antioxidant activity
- Anti obesity
- Antibacterial
- Anticancer
- Antidiabetic
- Antifertility
- Hepatoprotective
- Hypolipidemic
- Litholytic
- Trypsin and α-amylase inhibitor.
- Hyperglycaemic activity
- Low nitrate stress
- Anti-tubular activity
- Atypical anti-psychotic
- Anti-fungal activity

5. MATERIALS AND METHODS

1. PLANT COLLECTION AND AUTHENTICATION:

The seeds of *Phaseolus vulgaris Linn* were collected from farmers of Ooty, Tamil Nadu, India. They were authenticated by Dr. Stephen Ph.D., Professor, Dept. of Botany, American College, Madurai.

2. PREPARATION OF EXTRACTS *Phaseolus vulgaris Linn*:

AQUEOUS EXTRACT

Coarse powder of *Phaseolus vulgaris Linn* seeds (300 grams) were extracted by cold maceration using water for 15 days. The extract was concentrated by surface evaporation followed by vacuum drying. Dry powder was weighed and sored in air-tight containers for further Phytochemical and pharmacological studies.

ETHANOLIC EXTRACT

Coarse powder of *Phaseolus vulgaris Linn seeds* was extracted with 250 ml of ethanol by hot percolation method using soxhlet apparatus. The extraction was carried out for 72 hours. After extraction, the solvent was distilled out to obtain a concentrated extract. Then the concentrated extract was vacuum dried and the dry extract was stored in an air tight container for further Phytochemical and pharmacological studies.

3. IDENTIFICATION OF PHYTOCONSTITUENTS OF EXTRACTS :

I. Preliminary phytochemical screening: [68, 69, 70]

Preliminary phytoconstituents present in the ethanol extract and aqueous extract of *Phaseolus vulgaris* were identified based on the following chemical tests.

1**. Tests For Carbohydrates:**

a. Molisch's test:

To 2-3 ml of aqueous extract, added few drops of alpha naphthol solution in alcohol, shaken and then added concentrated sulphuric acid from sides of test tube. A brown purple ring formed at the junction of the two liquids indicates the presence of sugars.

b. Fehling's test:

Mixed 1 ml Fehling's solution A and 1ml of Fehling's solution B and boiled for 1 minute, added equal volume of test solution and heated on boiling water bath for 5-10 mins. Formation of brick red precipitate confirms the presence of sugars.

c. Benedict's test:

Mixed equal volume of Benedict's reagent and test solution in a test tube, heated in boiling water bath for 5 mins. Formation of brick red precipitate confirms the presence of sugars.

d. Barfoed's test:

Mixed equal volume of Barfoed's reagent and test solution. Heated for 1-2 mins in boiling water bath and cooled. An orange red precipitate confirms the presence of sugars.

2. **Tests for glycosides:**

Heated on a water bath and the hydrolysate was subjected to Legal, Keller Killiani, Borntrager's and modified Borntrager's test to detect the presence of glycosides.

a. Legal test:

To the hydrolysate, 1 ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. A blood red colour indicates the presence of glycosides.

b. Borntrager's test:

The hydrolysate was treated with chloroform and the chloroform layer was separated. To this, equal quantity of dilute ammonia solution was added. A light pink colour at the interface between two liquids indicates the presence of glycosides.

3. **Test for alkaloids**:

a. Mayer's test:

Methanolic extract was treated with drops of hydrochloric acid and filtered. The filtrate was treated with Mayer's reagent. Yellowish buff colour indicates the presence of alkaloids.

b. Dragendroffs test:

Methanolic extract was treated with few drops of Dragendroff's reagent. Orange red precipitate indicates the presence of alkaloids.

c.Wagner's test:

Methanolic extract was treated with drops of hydrochloric acid and filtered' The filtrate was treated with Wagner's reagent. Reddish brown colour precipitate indicates the presence of alkaloids.

4. **Tests for flavonoids:**

a. A small quantity of solvent free methanolic extract was dissolved in alcohol separately and it was extracted with 10% sulphuric acid and cooled. Then it was extracted with diethyl ether and divided in to three portions in two separate test tubes for each extract. 1ml of sodium carbonate, 1ml of sodium hydroxide and 1ml of diluted ammonia solution were added to the first and second test tubes respectively. In each test tube development of yellow colour demonstrated the presence of flavonoids.

b. Ferric chloride test:

To a small quantity of the alcohol solution of extract few drops of neutral ferric chloride solution were added. Formation of blackish red colour demonstrated the presence of flavonoids.

c. Shinoda's test:

To the alcoholic solution of extract a small piece of magnesium ribbon and few drops of concentrated hydrochloric acid were added and heated, a magenta colour indicates the presence of flavonoids in methanol extract .

5. **Tests for proteins:**

a. Biuret test:

The extract was treated with equal volume of 40% of sodium hydroxide and 2 drops of 1% copper sulphate solution . Pink or purple colour indicates the presence of proteins.

b. Millon's test:

To the extract, few drops of Millon's reagent was added and heated. Appearance of red colour indicates the presence of proteins and free amino acids .

C. Ninhydrin test:

A small quantity of extract solution was boiled 0.2% solution of Ninhydrin. Blue colour indicates the presence of free amino acids.

6.**Tests for tannins:**

Small quantity of the extract was dissolved in distilled water, filtered and tested for the presence of phenolic compounds and tannins using the following reagents:

A. With dilute ferric chloride solution (5%) – development of greenish black coloration indicates the presence of tannins.
B. With 10% lead acetate solution – development of yellow colour precipitate indicates the presence of tannis.
C. With 10% aqueous potassium dichromate solution – development of yellowish brown precipitate indicates the presence of tannis.

7. **Test for saponins:**

Foam test:

To the extract, 20ml of distilled water and agitated in a graduated cylinder for15 minutes. The formation of about 1cm layer of foam indicates the presence of saponins.

8. **Tests for steroids and triterpenoids:**

A. Libermann - Burchard reaction:

Small quantities of solvent free methanol extract were separately dissolved in 1ml chloroform and 1ml of acetic anhydride was then added followed by 2ml of concentrated sulphuric acid. A reddish violet ring at the junction of the two layers indicates the presence of triterpenoids and steroids.

b.Salkowski's test:

Concentrated sulphuric acid was added to a chloroform solution of the extract (10mg of extract in 1ml of chloroform), a reddish blue colour in the chloroform layer and green fluorescence in acid layer, suggests the presence of steroids.

9. **Test for sterols :**

When the extracts were treated with 5% potassium hydroxide solution, appearance of pink colour indicates the presence of sterols.

10. **Test for phenols:**

When the extracts were treated with neutral ferric chloride solution, appearance of violet colour indicates the presense of phenols. When the extracts were treated with 10% sodium chloride solution, the appearance of cream colour indicates the presence of phenol.

11. **Test for Terpenoids:**

About 0.5 g of plant extract in separate test tube was taken with 2 ml of chloroform; 5 ml of concentrated sulphuric acid was carefully added to form a layer and observed for presence of reddish brown colour interface to show positive results for the presence of terpenoids.

II. TLC Study on the extracts: [74]

The ethanolic extract of *phaseolus vulgaris* (EEPV) was dissolved in ethanol. Then the solution was applied on Merck Aluminium plate pre coated with silica gel 60 F_{254} of 0.2-0.5 mm thickness. The plate was developed in Chloroform: Methanol: Distilled water: Toluene (8:1:0.5:0.5) solvent system. After visualization Rf values were calculated.

The aqueous extract of *phaseolus vulgaris* (AEPV) was dissolved in water. Then the solution was applied on Merck Aluminium plate pre coated with silica gel 60 F_{254} of 0.2-0.5 mm thickness. The plate was developed in Chloroform: Methanol: Distilled water: Toluene (8:1:0.5:0.5) solvent system. After visualization Rf values were calculated.

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

4. DETERMINATION OF PHYTOCONSTITUENTS OF EXTRACTS

I. Determination of total flavonoid content: [75]

Preparation of standard:

Standard solution was prepared by adding 10 mg of accurately weighed Quercetin in 10 ml of distilled water.

Preparation of sample:

10 mg of the accurately weighed AEPV and EEPV extracts were separately dissolved in 10 ml water and used for the estimation.

Procedure:

The total flavoniod content of the AEPV and EEPV was determined by using aluminium chloride colorimetric method. To an aliquot of 1 ml of extract (1mg /ml) or standard solutions of Quercetin (10,20,40,60,80.100, µg/ml) methanol was added separately to make up the solution upto 2ml. The resulting mixture was treated with 0.1ml of potassium acetate and 2.8 ml of distilled water. Shaken well and incubated at room temperature for 30 minutes. The absorbance was measured at 415nm against blank, where a solution of 2ml ethanol 0.1ml potassium aetate, 2.8ml distilled water and 0.1ml of aluminium chloride serve as blank solution. The total flavanoid content was determined from the standard quercetin calibration curve. And it was expressed as milligrams of Quercetin equivalents (QE) per gram of extract

II. Determination of total phenolic content: [75]

Preparation of standard:

Standard solution was preapared by adding 10mg of accurately weighed Gallic acid in 10 ml of distilled water

Prepartion of sample:

10mg of the accurately weighed AEPV and EEPV extracts were separately dissolved in 10ml ethanol and used for the estimation.

Procedure:

The total phenolic content of the AEPV and EEPV was determined by Folin Ciocalteau assay method. To an aliquot 100µg of AEPV or standard solution of Gallic acid (10, 20, 40, 60, 80,100µg/ml) added 0.5ml of Folin Ciocalteau reagent and made into 2ml with distilled water and the mixture is incubated for 5min at room temperature . 0.1ml of 20% Sodium Carbonate and 0.9ml of distilled water were added to make the final solution to 3ml. It was incubated for 30 ins in dark to complete the reaction .After that absorbance of the mixture was measured at 725nm against blank. Distilled water was used as reagent blank. The tests were performed in triplicate to get mean the values. The total phenolic content was found out from the calibration curve of Gallic acid. And it was expressed as milligrams of Gallic acid equivalents (GAE) Per gram of extract.

5. ***IN VITRO*** **ANTIOXIDANT STUDIES ON THE EXTRACTS:**

1. Determination of Nitric Oxide Scavenging Assay: [71]

The activity was measured according to the modified method of Sreejayan and Rao, To 4ml of the extract having different concentrations (100-500 µg/ml), 1 ml of sodium nitroprusside (SNP) solution (5mM) was added and incubated for 2hr at 27˚C.An aliquot (2ml) of the incubation solution was removed and diluted with 1.2ml of Griess reagent (1% Sulfanilamide in 5% H_3PO_4 and 0.1% naphthylethylene diamine dihydrochloride). The absorbance of the chromophore was read at 550nm and compared with standard, Ascarbic Acid.

$$\text{Nitric oxide scavenging activity (\%)} = \frac{(\text{Abs control} - \text{Abs sample})}{(\text{Abs control})} \text{x}100$$

Where, Abs (control): Absorbance of the control and Abs (sample): Absorbance of the extracts/standard.

II. Determination of Reducing Power Assay: [72]

Various concentrations of the plant extracts in corresponding solvents were mixed with phosphate buffer (2.5ml) and potassium ferricyanide (2.5ml) . This mixture was kept at 50˚C in water bath for 20 minutes. After cooling, 2.5ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10mins whenever necessary. The upper layer solution (2.5ml) was mixed with distilled water (2.5ml) and a freshly prepared ferric chloride solution (0.5ml) . The absorbance was measured at700nm. Control was prepared in similar manner

excluding samples. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.

III. Determination of Lipid Peroxidation Assay: [73]

Egg homogenate (10% in 1.15% potassium chloride, v/v) 0.1 ml of extract/standard (100-500µg) were mixed in a test tube and the volume was made up to 2ml, by adding distilled water . Finally, 0.5 ml $FeSO_4$ (0.07M) was added to the above mixture and incubated for 30 minutes, to induce lipid peroxidation . Thereafter, 0.5ml of 20% acetic acid (pH 3.5) and 0.5ml of 0.8% TBA (w/v) (prepared in 1.1% sodium dodecyl sulphate) and 0.5ml 20% TCA were added, vortexed, and then heated in a boiling water bath for 60mins. After cooling, 5.0ml of 1-butanol was added to each tube and centrifuged at 3000 rpm for 10mins. The absorbance of the organic upper layer was measured at 532nm. For the blank 1.0ml of distilled water was used in place of the extact.

$$AI = (1 - T/C) \times 100$$

Where T = absorbance of Test, C = absorbance of fully oxidized control.

6. PHARMACOLOGICAL SCREENING FOR ANTILITHIATIC ACTIVITY:

Experimental Animals:

Wistar rats of either sex, weighing 150-200g were maintened in Animal house. The selected animals were grouped and housed in polypropylene cages in standard environmental conditions at 23 ± 2^0 with 12:12 Hr dark and light cycle. The animal had free acess to food and water *ad libitum*. All animals were housed standard hygienic laboratory condition one week prior to testing.

The experiments on animals were conducted in accordance with the Experimental protocols duly approved by Institutional Animal Ethical Committee.

Proposal No: (IAEC/ T.VINCIYA/ TNMGRMU/ M.PHARM/ KMCP/ 06/ 2017-2018)

Experimental Design: [9]

Rats were divided into nine groups of three animals each (n = 3). CaOx stones were induced in rats by administering 0.75% v/v of EG and 1 % w/v of AC in drinking water for 15 days. Cystone (750 mg/kg) was used as a standard drug, and extract of *phaseolus vulgaris* (EPV) was administered at doses of 200 and 400 mg/kg. Both preventive and curative effects of EPV were evaluated. All groups were maintain on commercial pellet diet for 28 day.

The treatment schedule was planned as follows:

Group I: Normal (untreated)

Group II: Preventive control (EG + AC + vehicle from day 1 to 15)

Group III: Preventive standard (EG + AC + cystone 750 mg/ kg, orally from day 1 to 15)
Group IV: Preventive low dose (EG + AC + EPV 200 mg/kg, orally from day 1 to 15)

Group V: Preventive high dose (EG +AC + EPV 400 mg/kg, orally from day 1 to 15)

Group VI: Curative control (EG + AC from day 1 to 15, vehicle from day 16 to 30)

Group VII: Curative standard (EG + AC from day 1 to 15; cystone 750 mg/kg, orally from day 16 to 30)

Group VIII: Curative low dose (EG + AC from day 1 to 15, EPV 200 mg/kg, orally from day 16 to 30)

Group IX: Curative high dose (EG + AC from day 1 to 15, EPV 400 mg/kg, orally from day 16 to 30)

ASSESSMENT OF ANTIUROLITHIATIC ACTIVITY: [25]

Urine collection and analysis :

At the end of the 14th and 28th day, the animals were kept separately in metabolic cages for 24 hours for urine collection. Animals had free access to drinking water during the urine collection period.

The collected urine samples were measured for the following parameters:

Urine volume, Creatinine, Calcium, Magnesium, Oxalate, Phosphate.

Urine volume: measured using the measuring cylinder and reported per ml.

Calcium and magnesium: determined by Colourimetric method.

Oxalate: determined by hodgkinson and Williams method.

Phosphate: using fiske and subbarow method.

Serum Analysis:

Blood samples were collected from the retro-orbital venous plexus and serum was separated by centrifuging at 1500 rpm for 15 min and used for the estimation of uric acid, creatinine, and Blood urea nitrogen (BUN) using ERBA diagnostic kits according to the manufacturer's instructions. Fully automated autoanalyzer (Erba EM-200, Transasia Bio-medicals Ltd, Mumbai) was used for the estimations.

E. Histopathological examination: [9]

Immediately after blood sampling, animals were sacrificed by cervical dislocation under ether anesthesia. No animal died prior to this experimental end point. The two kidneys from each rat were immediately dissected out and rinsed with Phosphate buffered saline (PBS) to remove excess blood. kidneys from all groups were fixed in 10% neutral buffered formalin for 72h at least, washed, dehydrated, and embedded in paraffin. Sections of 5µm thickness were stained with Hematoxylin and Eosin (H&E).

Statistical Analysis:

Datas's of all the parameters were analysed using the Graph pad 7.0 software. Analysis of Variance (ANOVA); one way ANOVA followed by Dunnetts t test was performed. The values were expressed as Mean ± SEM. P value <0.05, $p<0.01$ was considered as significant.

6. RESULTS AND ANALYSIS

1. EXTRACTIVE YIELD OF *Phaseolus vulgaris linn*:

The percentage yield of ethanolic and aqueous extract of seeds of *Phaseolus vulgaris linn* was found to be

Ethanolic extract: 8.24 % w/w.

Aqueous extract: 6.32 % w/w.

2. IDENTIFICATION OF PHYTOCONSTITUENTS OF EXTRACTS:

1. Preliminary Phytochemical screening of *Phaseolus vulgaris*:

Preliminary Phytochemical screening of *Phaseolus vulgaris*, revealed the presence of following phytoconstituents.

Table No: 1. Results of preliminary Phytochemical screening of *Phaseolus vulgaris*

S No	Constituents	Observation	
		Ethanol	Aqueous
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Proteins & amino acids	+	+
4	Steroids	+	+
5	Phenols	+	+
6	Tannins	+	+
7	Flavonoids	+	+
8	Glycosides	+	+
9	Saponins	–	+
10	Terpenoids	+	+

(-) indicates the absence of compound

(+) indicates the presence of compound

II. Thin Layer Chromatography of *Phaseolus vulgaris*:

TLC study was carried out for the separation and identification of phytoconstituents in *Phaseolus vulgaris*, after development visualization was done with Iodine. Rf values werecalculated.

Aqueous extract **Ethanolic extract**

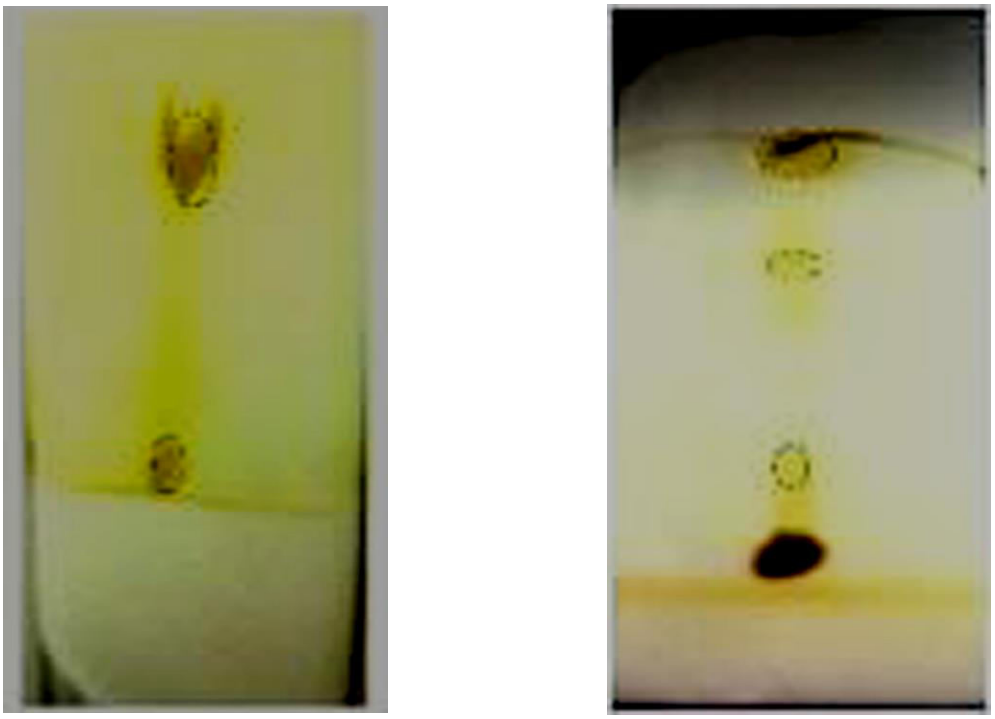


Figure No: 6. TLC Identification of phytoconstituents in AEPV and EEPV

Table No: 2. Results of TLC screening of *phaseolus vulgaris*

Aqueous extract		Ethanolic extract	
No of Spot	**R_f Value**	**No of Spot**	**R_f Value**
1	0.38	1	0.16
2	0.83	2	0.41
		3	0.55
		4	0.88

3. DETERMINATION OF PHYTOCONSTITUENTS OF EXTRACTS:

1. Determination of Total Flavonoid content:

Table: 3 Determination of Total Flavonoid content

Sample	Concentration µg/ml	OD value
Standard (Quercetin) 1mg/ml	10	0.03
	20	0.09
	40	0.26
	60	0.5
	80	0.78
	100	1.05
AEPV 1 mg/ml	100	0.04
	100	0.05
	100	0.04
EEPV 1 mg/ml	100	0.06
	100	0.04
	100	0.04

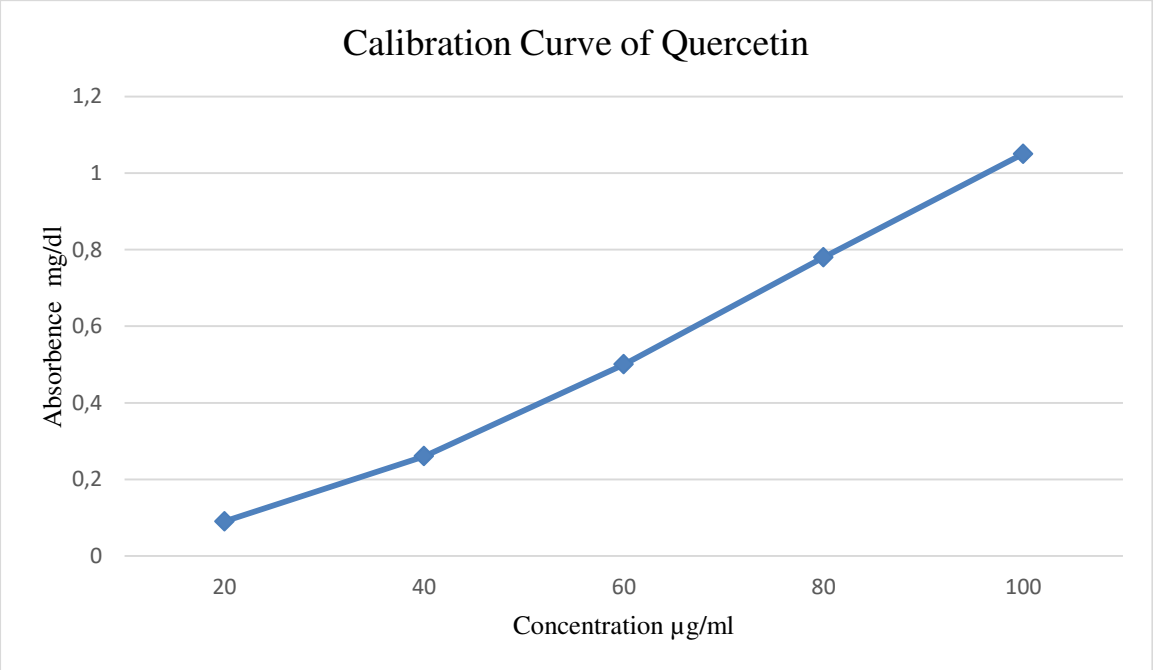


Figure No: 8 Determination of Total Flavonoid content

Total Flavonoid Content of AEPV was found to be 8.81 mg Quercetin Equivalents / g plant Extract.

Total Flavonoid Content of EEPV was found to be 9.18 mg Quercetin Equivalents / g plant Extract.

II. Determination of Total Phenolic content:

Table No: 4 Determination of Total Phenolic content:

Sample	Concentration µg/ml	OD value
Standard (Gallic Acid) 1mg/ml	10	0.09
	20	0.12
	40	0.21
	60	0.34
	80	0.40
	100	0.50
AEPV 1 mg/ml	100	0.13
	100	0.13
	100	0.11
EEPV 1 mg/ml	100	0.15
	100	0.19
	100	0.13

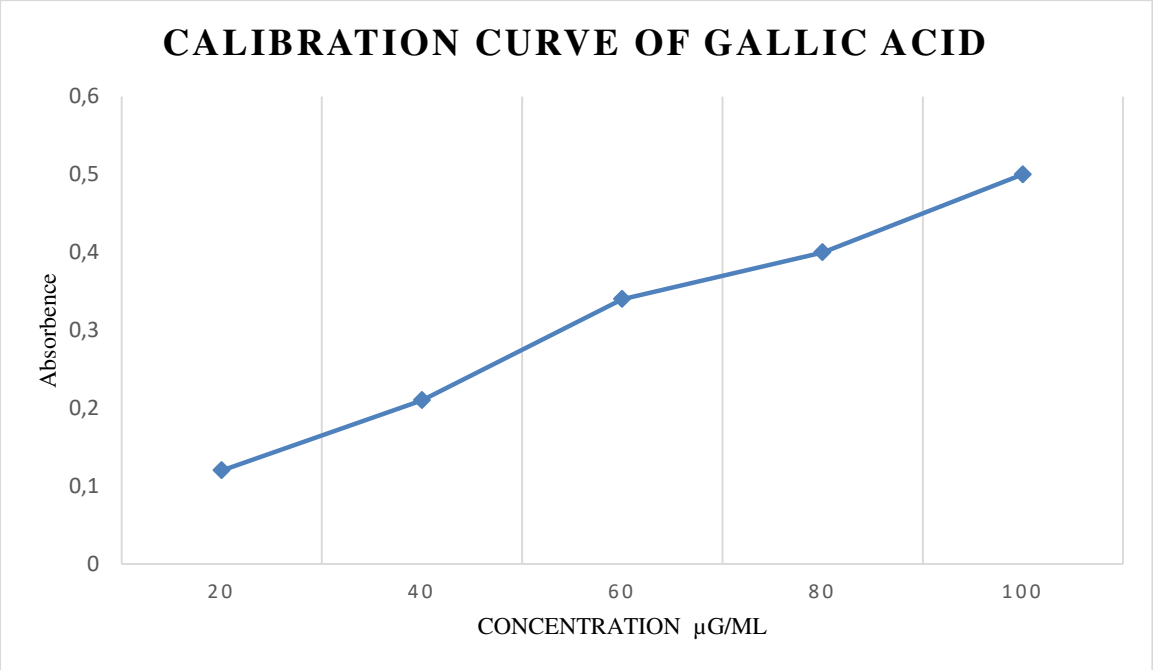


Figure No: 9 Determination of Total Phenolic content

Total Phenolic Content of AEPV was found to be 30.10 mg Gallic acid Equivalents / g plant Extract.

Total Phenolic Content of EEPV was found to be 42.22 mg Gallic acid Equivalents / g plant Extract.

4. *IN VITRO* ANTI OXIDANT STUDIES ON THE EXTRACT:

1. Determination of Nitric Oxide Scavenging Assay:

EEPV: Ethanolic extract of*Phaseolus vulgaris*

AEPV: Aqueous extract of *Phaseolus vulgaris*

Table 5. Determination of Nitric Oxide Scavenging Assay

S. no	Concentration µg/ml	% of Inhibition			IC 50 Value (µg/ml)		
		Ascorbic Acid	EEPV	AEPV	Ascorbic Acid	EEPV	AEPV
1	200	86.38±1.32	23.25±1.77	59.04±2.34	98.26	651	195
2	400	90.33±1.56	40.69±1.02	63.33±1.09			
3	600	91.41±1.52	47.28±1.55	84.76±0.62			
4	800	92.93±1.76	64.72±1.02	87.37±0.23			
5	1000	93.94±1.49	72.85±1.404	91.41±0.41			

Values are mean ± SEM of 3 replicates

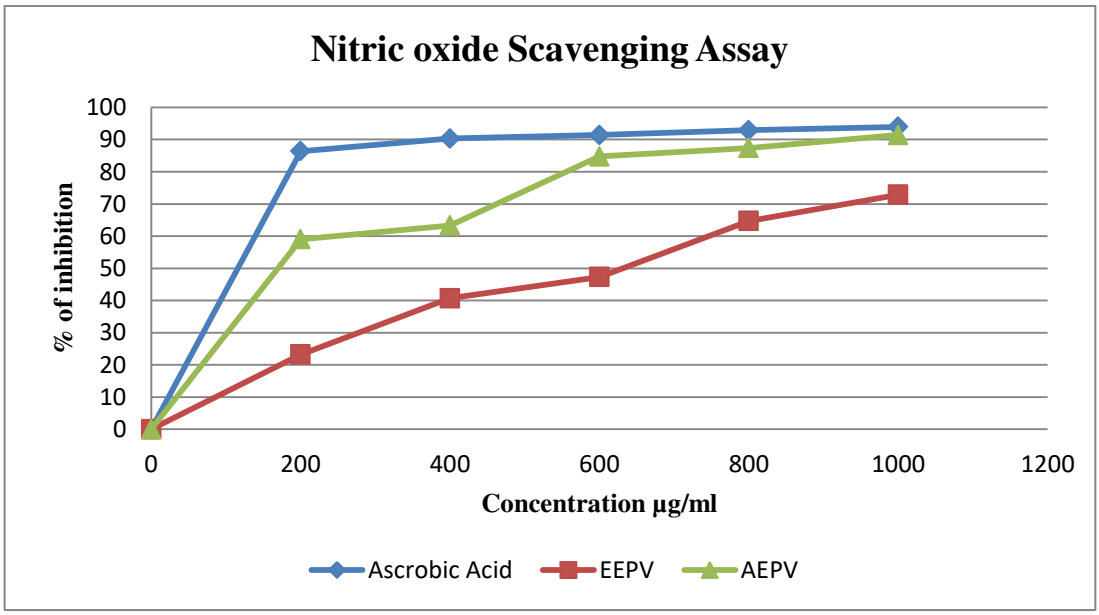


Figure No: 9 Determination of Nitric Oxide Scavenging Assay

II. DETERMINATION OF REDUCING POWER ASSAY:

Table No: 6. Determination of Reducing Power Assay:

S. No	Concentration µg/ml	OD value		
		Ascorbic Acid	EEPV	AEPV
1	200	1.23 ± 0.06	0.18 ± 0.008	0.47 ± 0.01
2	400	1.61 ± 0.04	0.21 ±0.01	0.60 ± 0.02
3	600	1.68 ±0.04	0.41 ± 0.01	0.94 ± 0.03
4	800	1.77 ± 0.07	0.64 ±0.02	1.19 ±0.04
5	1000	1.80 ± 0.08	1.04 ±0.04	1.41 ± 0.01

Values are mean ± SEM of 3 replicates

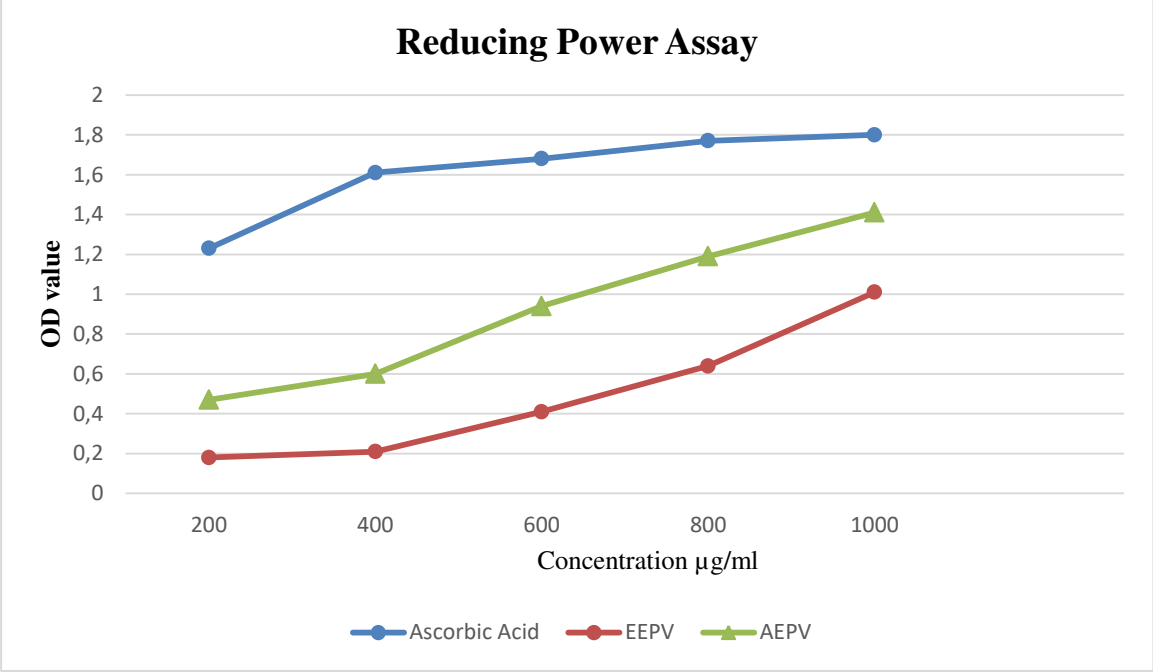


Figure No: 10 Determination of Reducing Power Assay

III. DETERMINATION OF LIPID PEROXIDATION ASSAY:

Table No: 7. Determination of Lipid Peroxidation Assay:

S. no	Concentration µg/ml	% of Inhibition			IC 50 Value (µg/ml)		
		Ascorbic Acid	EEPV	AEPV	Ascorbic Acid	EEPV	AEPV
1	200	70.5±1.32	25.99±1.77	21.71±2.9	171.66	700.66	732.22
2	400	77.31±1.62	38.09±1.17	31.81±0.87			
3	600	82.35±1.21	46.02±0.81	43.93±1.57			
4	800	86.55±1.45	55.55±1.58	54.28±2.20			
5	1000	90.76±1.68	60.29±1.20	60.09±1.10			

Values are mean ± SEM of 3 replicates

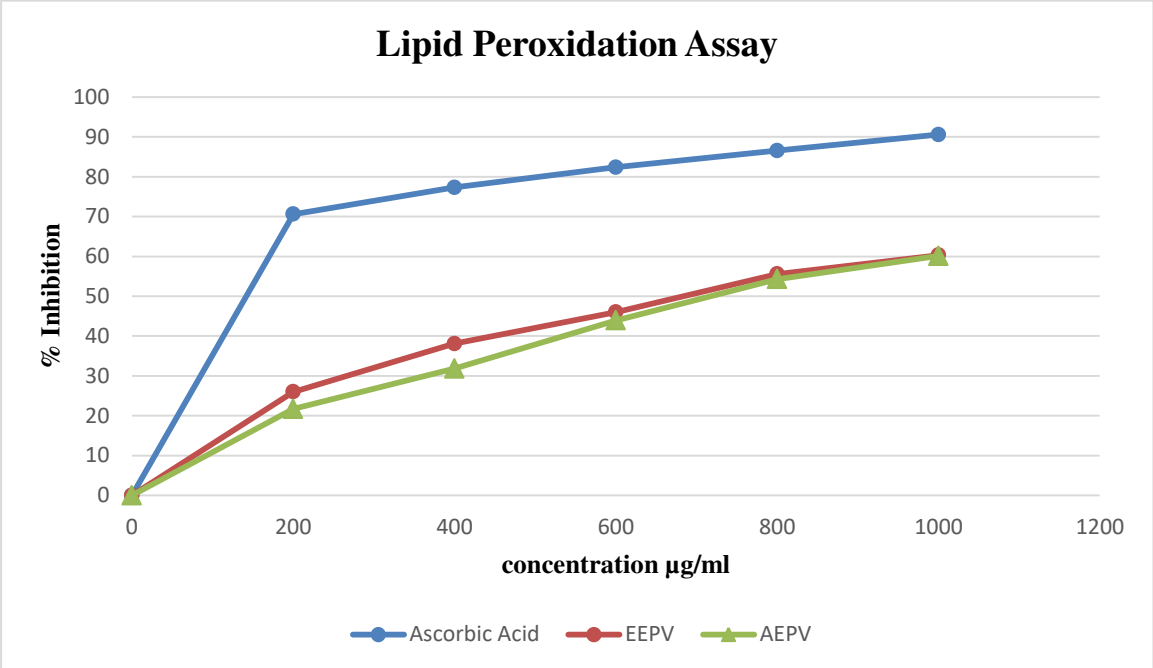


Figure No: 11. Determination of Lipid Peroxidation Assay

5. PHARMACOLOGICAL STUDIES

A. PREVENTIVE STUDY

Effect of AEPV on Urine Volume and Urine biochemical parameters on 14th day

Table No: 8. Effect of AEPV on Urine Volume and Urine biochemical parameters on 14th day

Group	URINE ANALYSIS				
	Urinary volume (ml/24 h)	Calcium (mg/dl)	Oxalate (mg/dl)	phosphate (mg/dl)	Magnesium (mg/dl)
Normal	9.71±0.63	4.48±0.166	1.15±0.434	5.61±0.25	1.44±0.23
Lithiatic control (un treated)	4.10±0.88	7.465±0.357	3.06±0.042	8.58±0.24	0.65±0.09
Positive control (cystone treated)	9.16±1.04*	6.029±0.09*	1.38±0.35**	6.64±0.26**	1.26±0.03**
AEPV Low dose(200mg/kg)	5.91±0.40ns	5.82±0.27*	1.37±0.33**	6.42±0.06**	1.21±0.06**
AEPV high dose (400mg/kg)	7.33±0.76*	6.17±0.08*	1.41±0.34**	6.72±0.18**	1.38±0.08**

Values are expressed as Mean ± SEM. *, **, ***, ns - Mean values are significantly different when compared with lithiatic control mean values at P<0.05, P<0.01, P<0.001and non-significant respectively

Effect of AEPV on Urine Volume and Urine biochemical parameters on 14th day was

1. Urinary volume in Lithiatic group is decreased, positive control group is increased. Urinary volume in AEPV in high dose is increased compared to the low dose.

2. Calcium, Oxalate, Phosphate level in Lithiatic group is increased, positive control and the extracts of AEPV in low dose and high dose may be decreased.

3. Magnesium level in Lithiatic group is decreased, positive control and the extracts of AEPV in low dose and high dose may be increased.

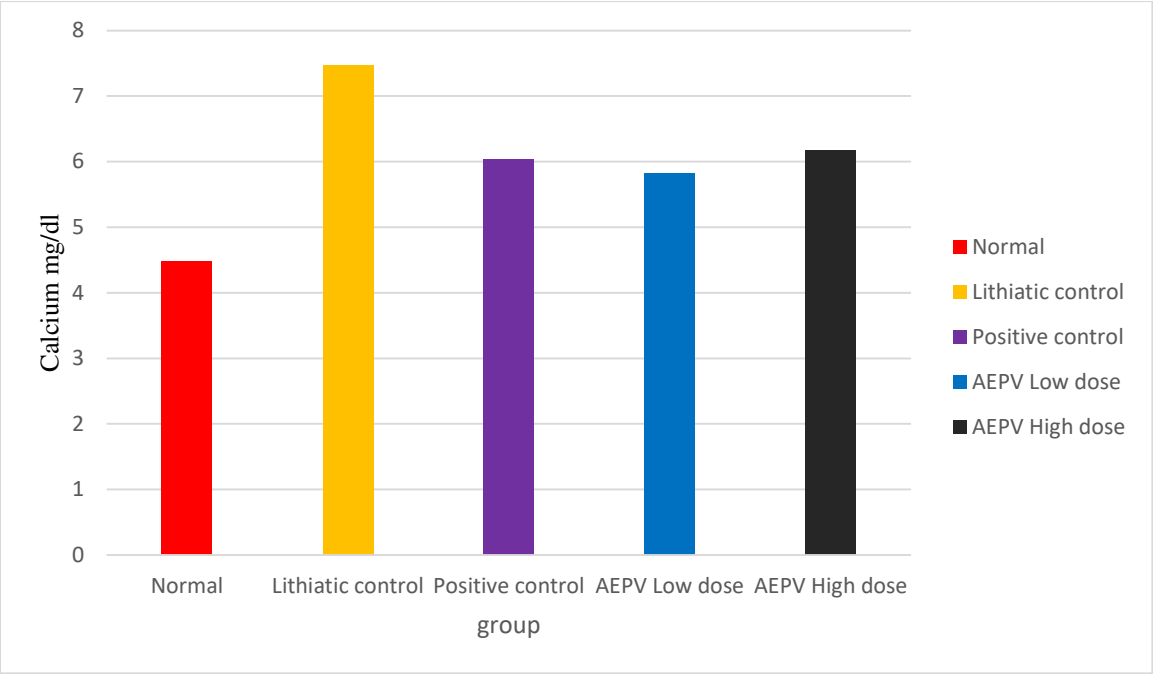


Figure No: 12. The 24 hr calcium concentration in rats urine on day 14

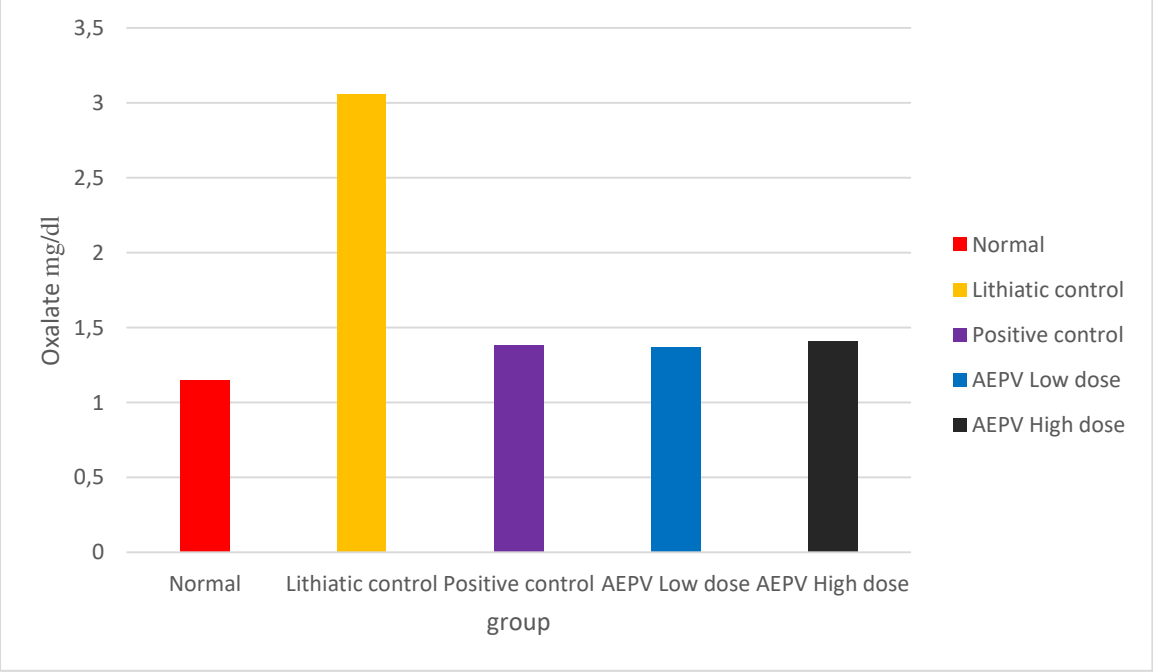


Figure No: 13. The 24 hr oxalate concentration in rats urine on day 14

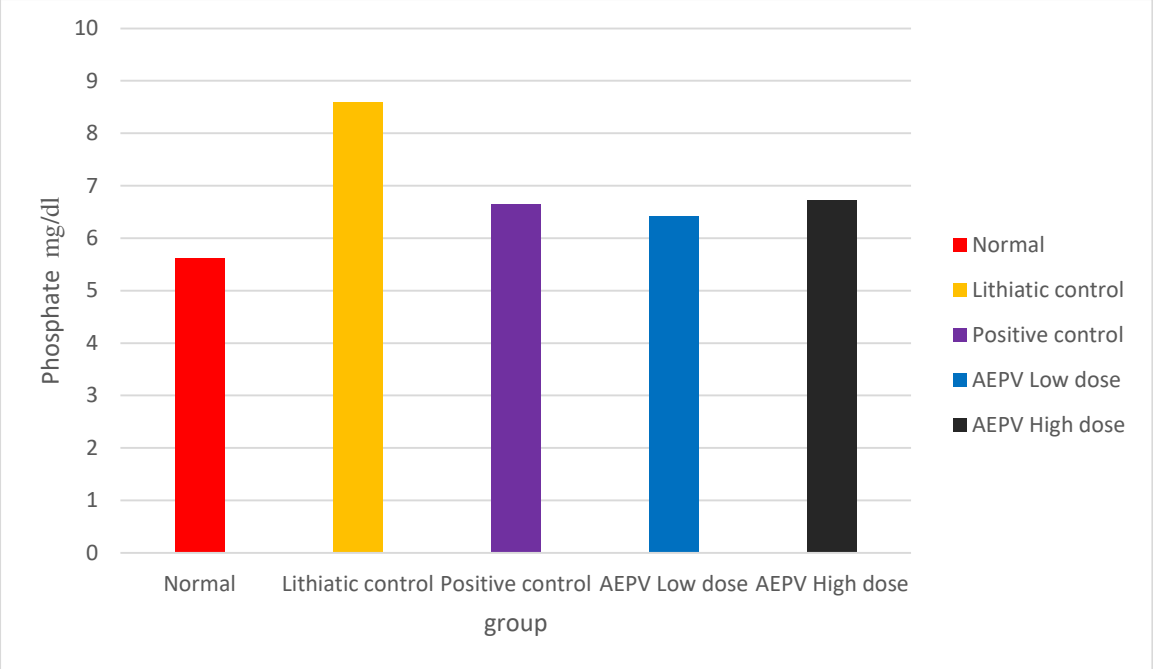


Figure No: 14. The 24 hr phosphate concentration in rats urine on day 14

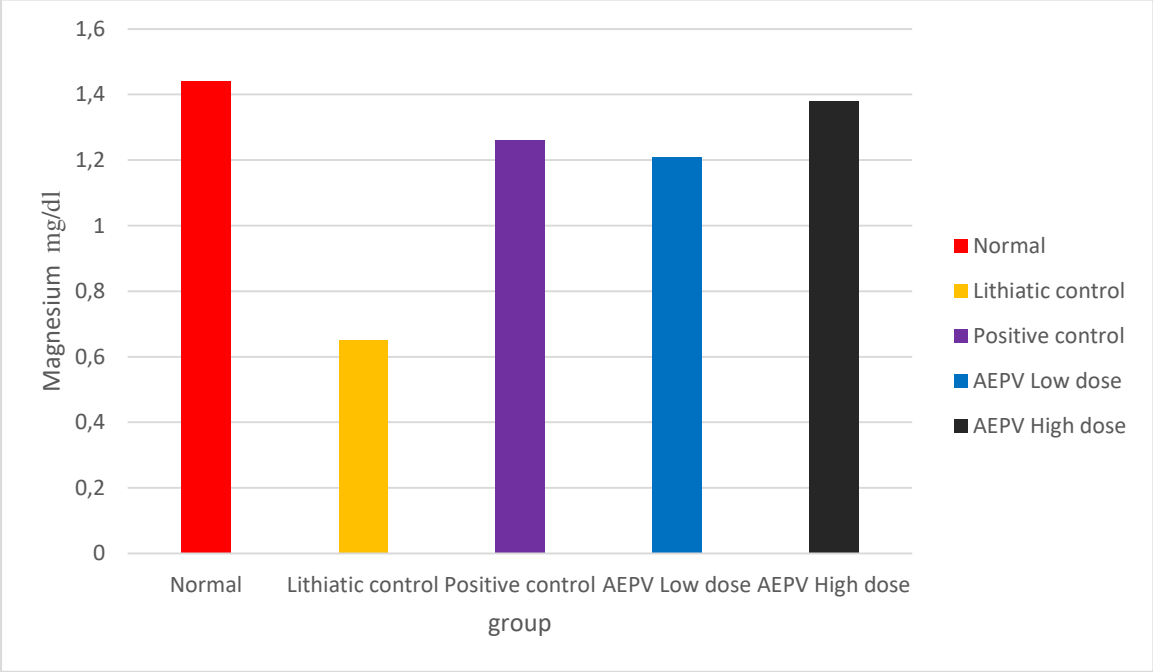


Figure No: 15. The 24 hr magnesium concentration in rats urine on day 14

Effect of AEPV on serum biochemical parameters on 14th day

Table No: 9. Effect of AEPV on serum biochemical parameters on 14th day

GROUP	SERUM ANALYSIS (mg/dl)		
	Creatinine	**Urea**	**BUN (Blood urea nitrogen)**
Normal	1.107±0.23	1.957±0.26	37.6±0.23
Lithiatic control (un treated)	2.06±0.16	5.46±0.63	46.17±2.49
Positive control (cystone treated)	0.723±0.014**	1.65±0.02**	31.19±4.04*
AEPV Low dose(200mg/kg)	0.93±0.058**	1.65±0.075**	34.37±3.25*
AEPV high dose(400mg/kg)	0.906±0.017**	1.83±0.06**	35.4±2.8*

Values are expressed as Mean ± SEM. *, **, ***, ns - Mean values are significantly different when compared with lithiatic control mean values at P <0.05, P<0.01, P<0.001 and non-significant respectively

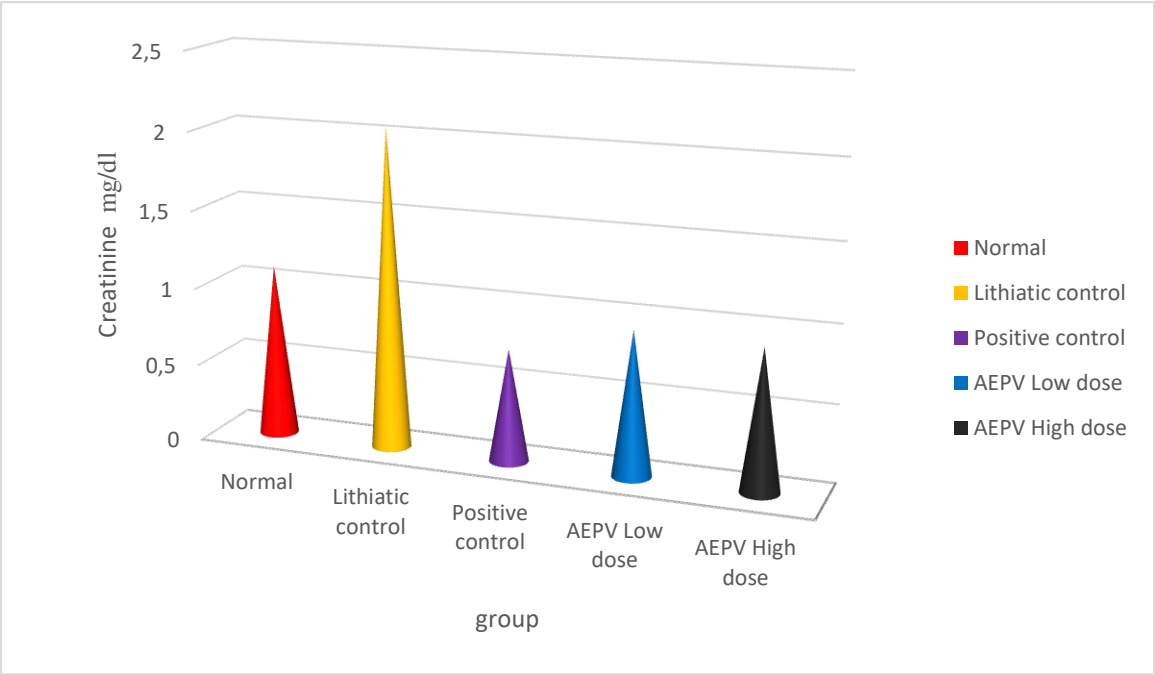


Figure No: 16. The creatinine excretion in rats blood on day 14

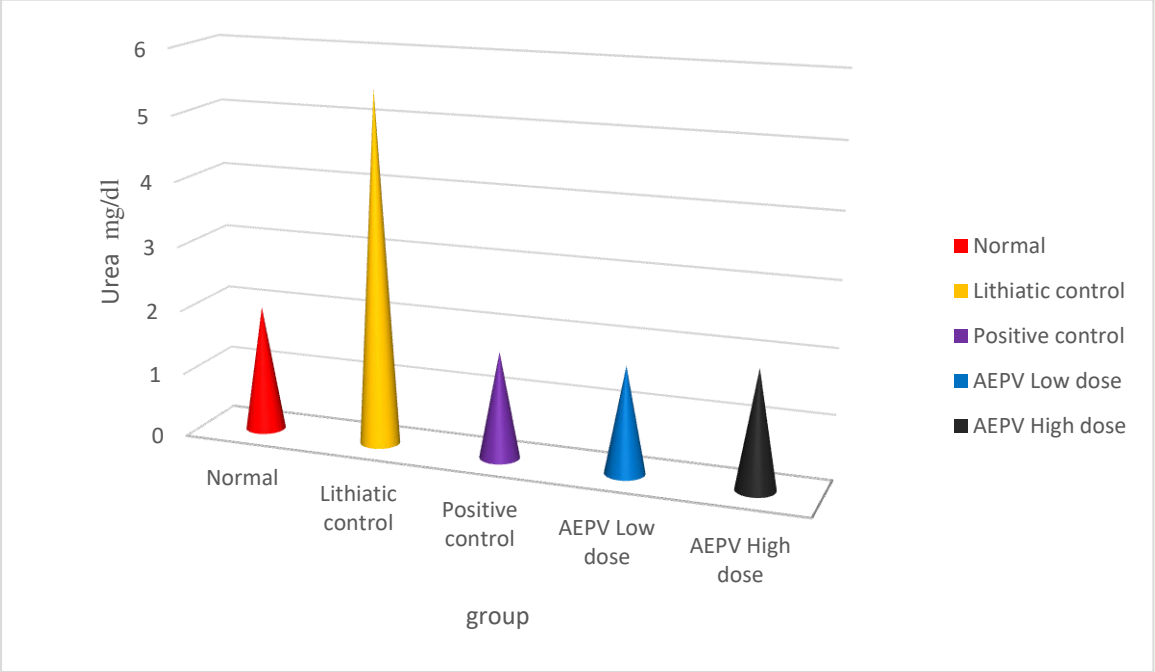


Figure No: 17. The urea excretion in rats blood on day 14

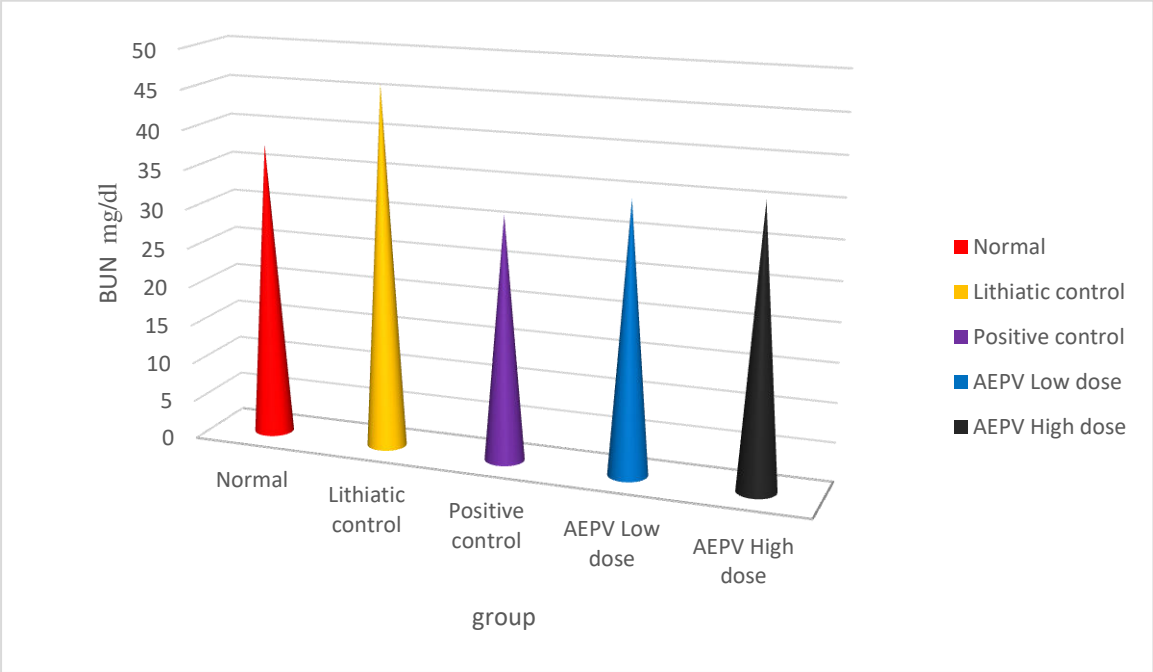


Figure No: 18. The BUN excretion in rats blood on day 14

Effect of EEPV on Urine Volume and Urine biochemical parameters on 14th day

Table No: 10. Effect of EEPV on Urine Volume and Urine biochemical parameters on 14th day

Group	URINE ANALYSIS				
	Urinary volume (ml/24 h)	**Calcium (mg/dl)**	**Oxalate (mg/dl)**	**phosphate (mg/dl)**	**Magnesium (mg/dl)**
Normal	7.89±0.12	5.7±0.24	0.34±0.013	5.83±0.39	0.96±0.59
Lithiatic control (un treated)	4.82±0.17	7.39±0.53	3.57±0.01	8.93±0.28	0.55±0.01
Positive control (cystone treated)	6.8±0.04***	4.28±0.37**	1.32±0.02*	6.68±0.11*	1.28±0.04*
EEPV Low dose(200mg/kg)	7.13±0.11***	5.04±0.23*	1.55±0.05*	7.03±0.07*	1.19±0.06*
EEPV high dose (400mg/kg)	7.11±0.20***	4.80±0.22*	1.41±0.01*	6.86±0.03*	1.52±0.04*

Values are expressed as Mean ± SEM. *, **, ***, ^{ns} - Mean values are significantly different when compared with lithiatic control mean values at P <0.05, P<0.01, P<0.001 and non-significant respectively

Effect of EEPV on Urine Volume and Urine biochemical parameters on 14th day was

1. Urinary volume in Lithiatic group is decreased, positive control group is increased. Urinary volume in EEPV in high dose is almost equal to the low dose.

2. Calcium,Oxalate, Phospahte level in Lithiatic group is increased, positive control and the extracts of EEPV in low dose and high dose may be decreased.

3. Magnesium level in Lithiatic group is decresed., positive control and the extracts of EEPV in low dose and high may be increased.

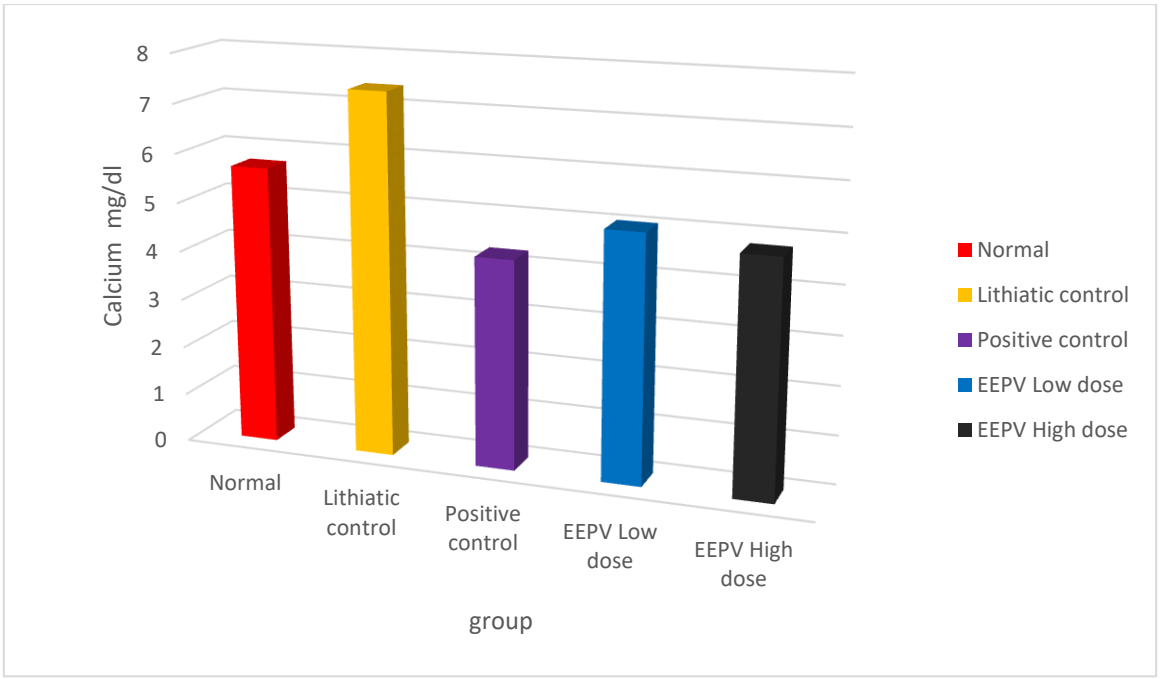


Figure No: 19. The calcium excretion in rats urine on day 14

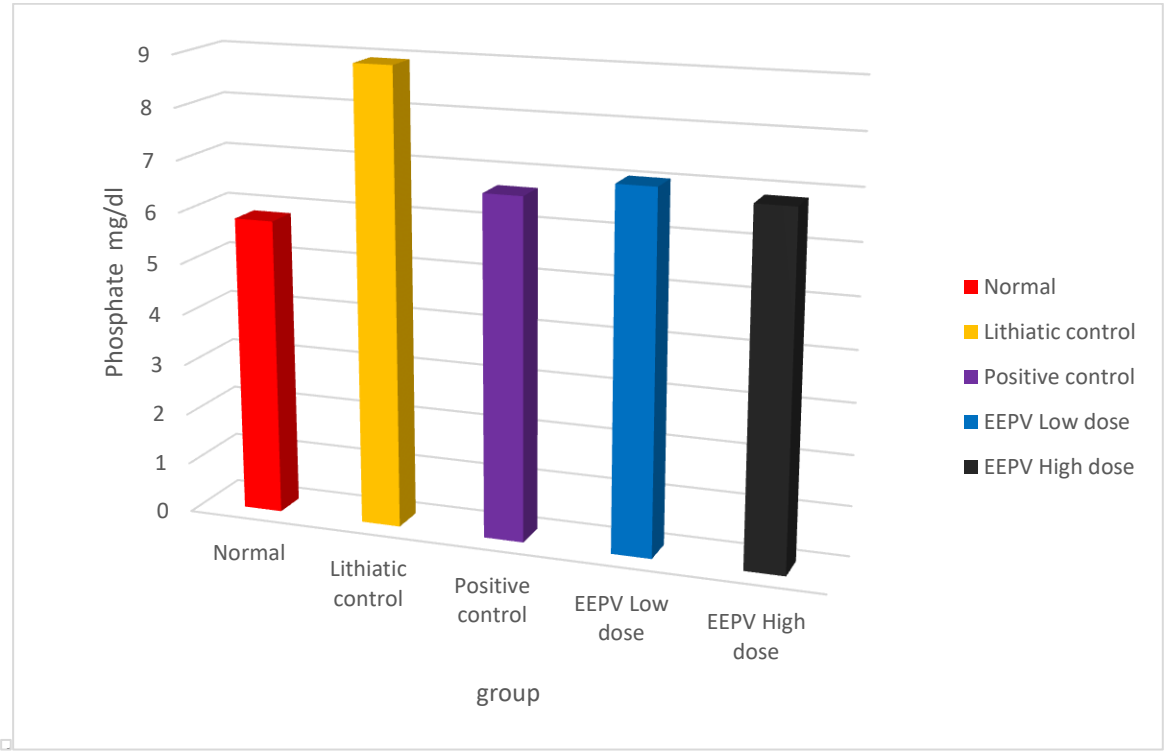


Figure No: 20. The phosphate excretion in rats urine on day 14

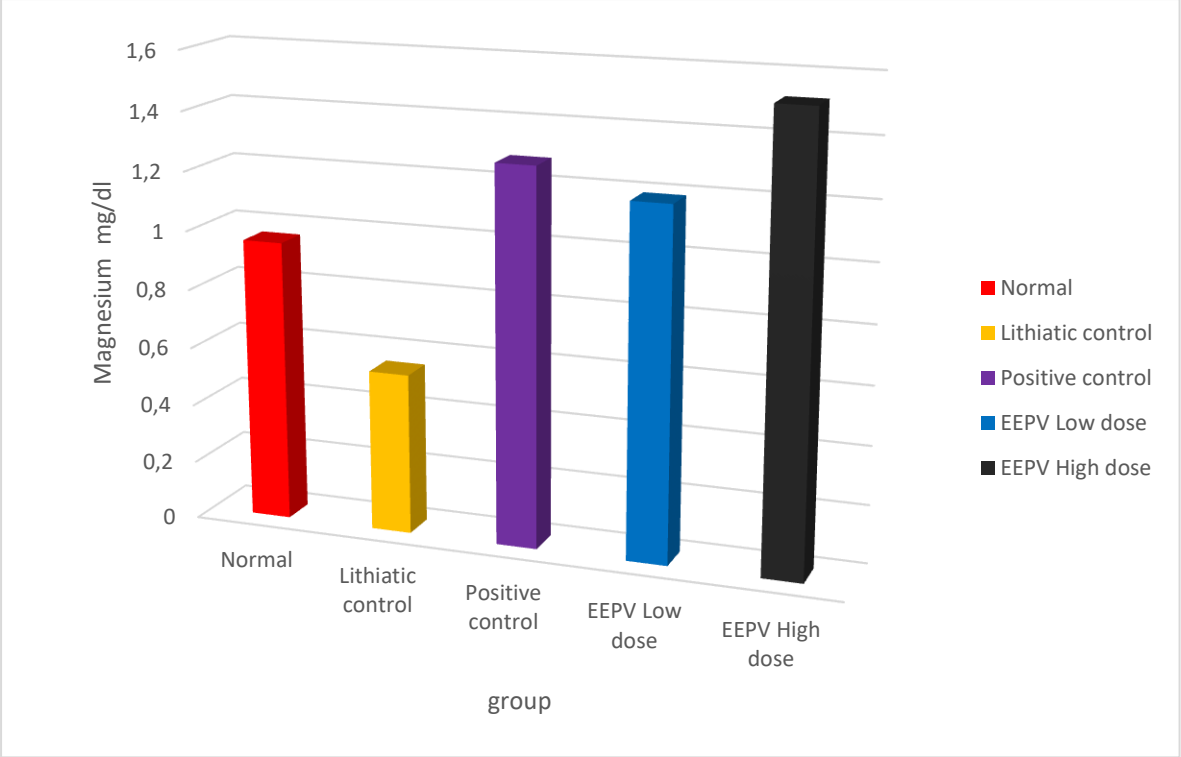


Figure No: 21. The magnesium excretion in rats urine on day 14

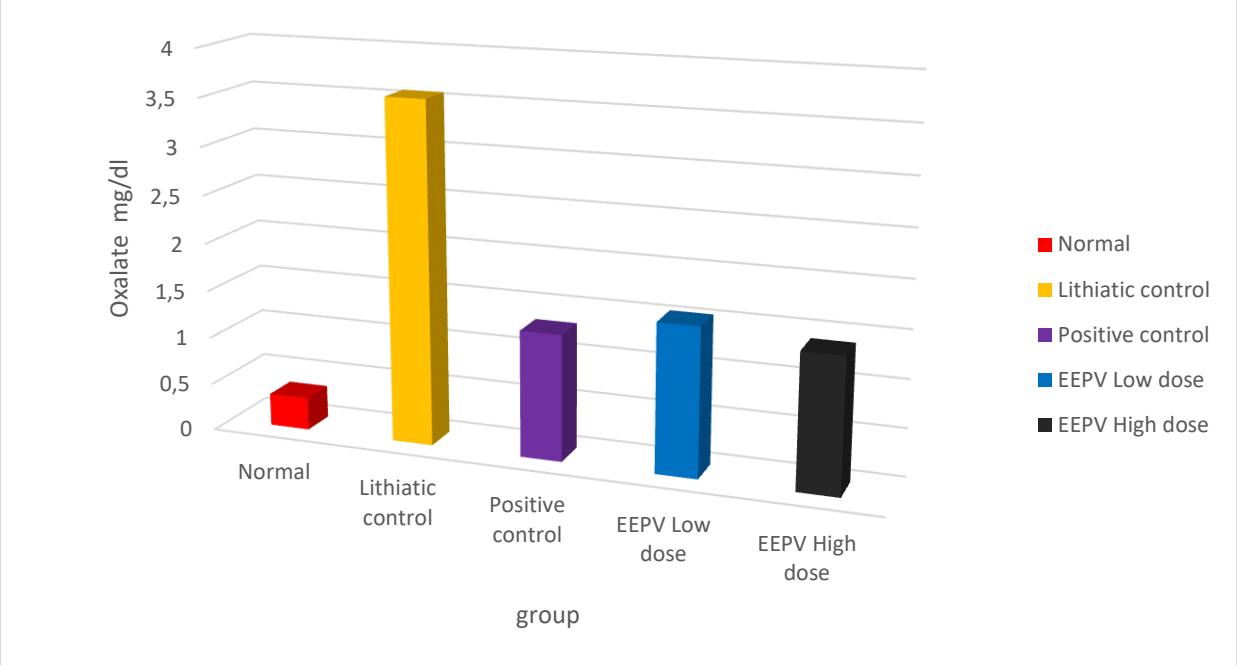


Figure No: 22. The oxalate excretion in rats urine on day 14

Effect of EEPV on serum biochemical parameters on 14th day

Table No: 11. Effect of EEPV on serum biochemical parameters on 14th day

Group	Serum Analysis (mg/dl)		
	Creatinine	**Uric acid**	**BUN (Blood urea nitrogen)**
Normal	0.75±0.01	1.49±0.07	37.62±0.15
Lithiatic control (un treated)	1.73±0.17	4.29±0.14	48.43±0.54
Positive control (cystone treated)	0.80±0.01*	1.62±0.03*	38.15±1.02*
EEPV Low dose(200mg/kg)	0.88±0.02*	1.92±0.02*	40.43±0.66*
EEPV high dose (400mg/kg)	0.82±0.02*	1.79±0.03*	39.48±0.49*

Values are expressed as Mean ± SEM. *, **, ***, ^{ns} - Mean values are significantly different when compared with lithiatic control mean values at P <0.05, P<0.01, P<0.001 and non-significant respectively.

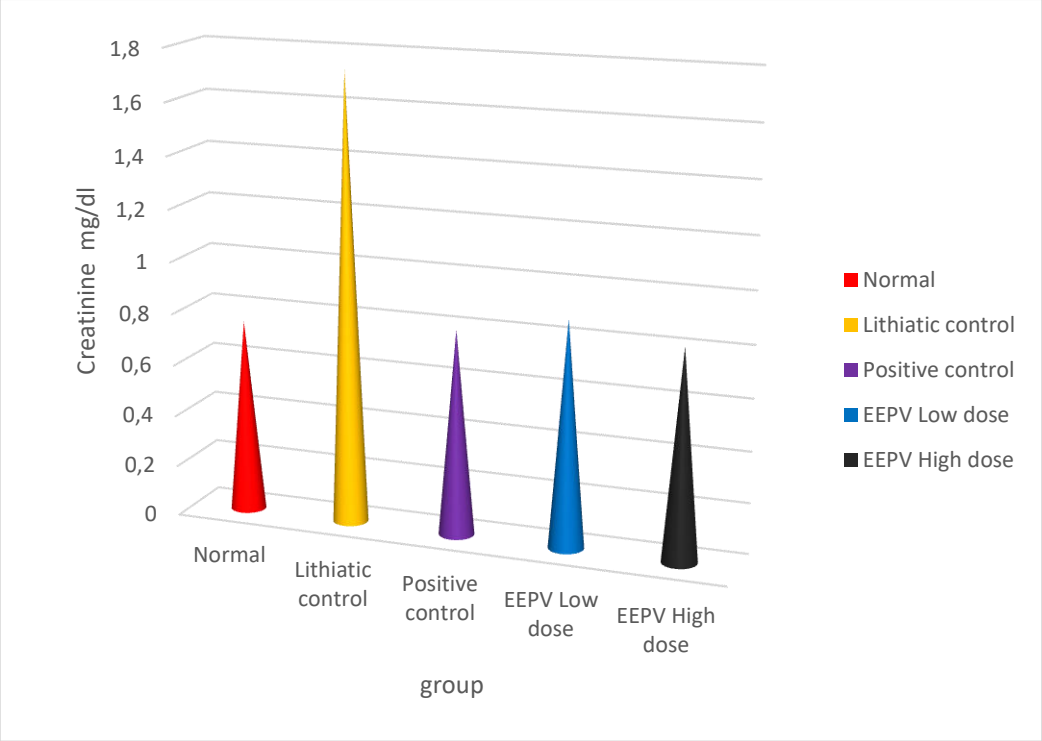


Figure No: 23. The creatinine excretion in rats blood on day 14

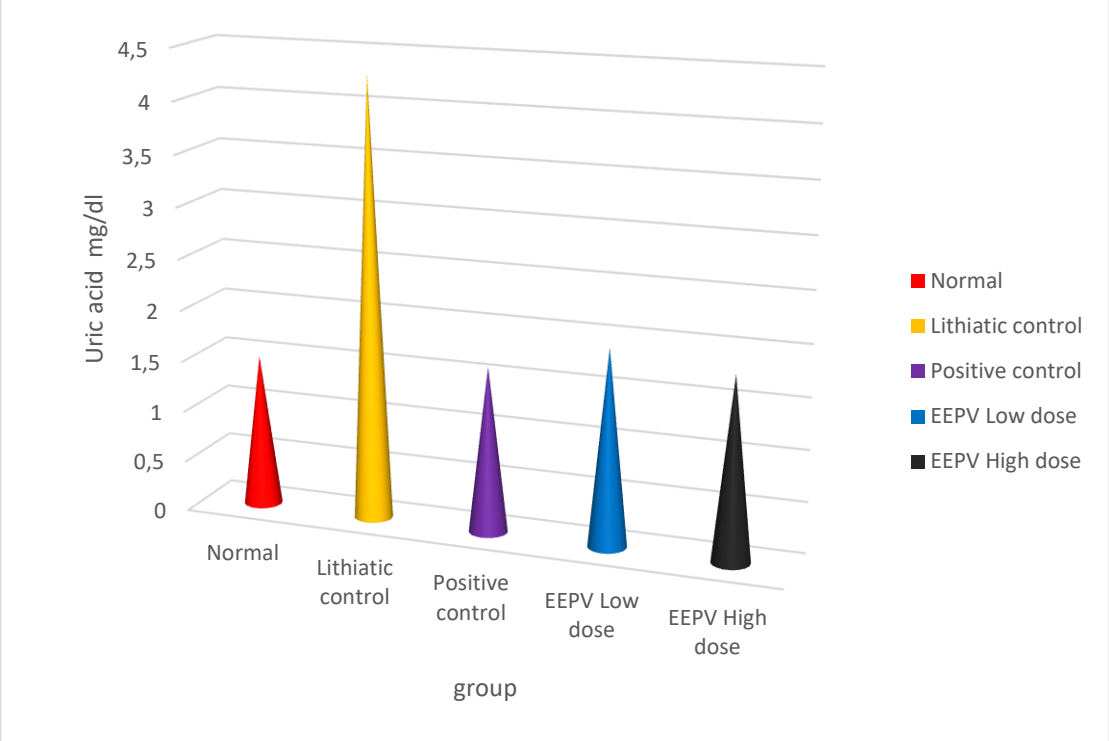


Figure No: 24. The Uric acid excretion in rats blood on day 14

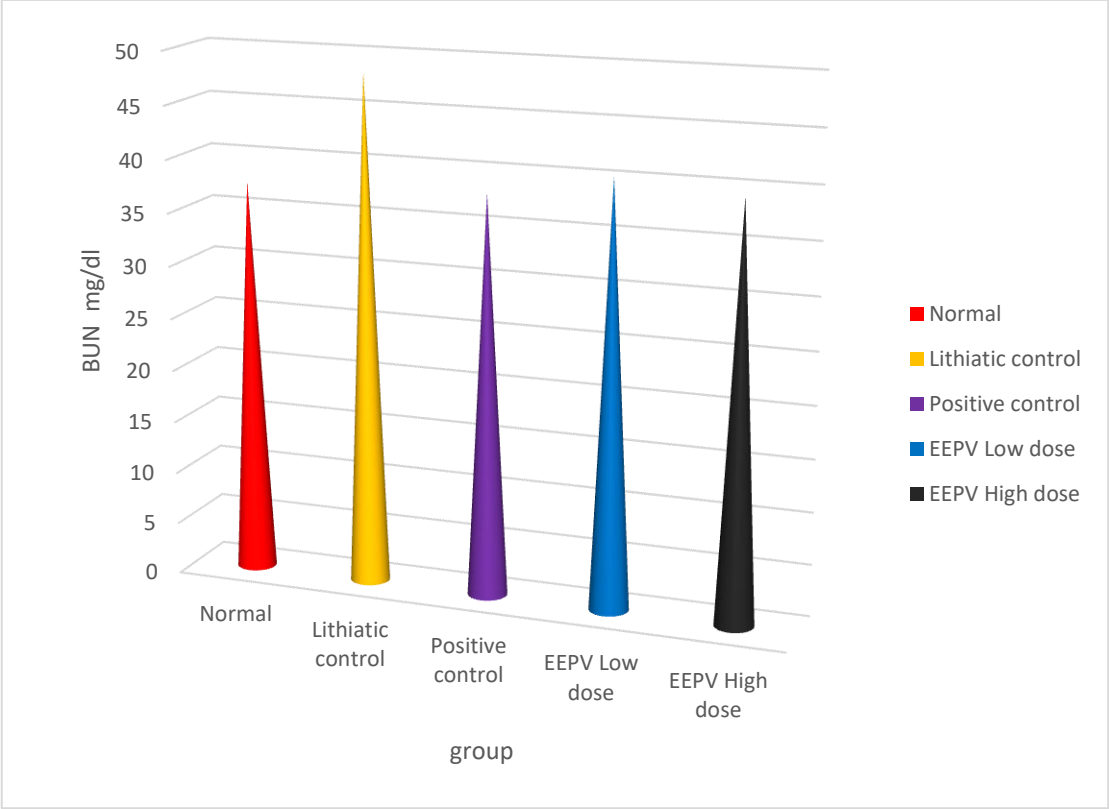


Figure No: 25. The BUN excretion in rats blood on day 14

Effect of AEPV on Urine Volume and Urine biochemical parameters on 28th day

Table No: 12. Effect of AEPV on Urine Volume and Urine biochemical parameters on 28th day

Group	URINE ANALYSIS				
	Urinary volume (ml/24 h)	**Calcium (mg/dl)**	**Oxalate (mg/dl)**	**phosphate (mg/dl)**	**Magnesium (mg/dl)**
Normal	5.95±0.13	4.14±0.3796	0.34±0.02	5.85±0.42	0.94±0.01
Lithiatic control (un treated)	3.61±0.14	7.26±0.515	2.47±0.11	8.57±0.419	0.69±0.012
Positive control (cystone treated)	5.08±0.1***	7.24±0.53	2.53±0.1	8.56±0.52	0.71±0.01
AEPV Low dose(200mg/kg)	4.16±0.17***	7.08±0.38	2.4±0.09	8.51±0.57	0.68±0.01
AEPV high dose (400mg/kg)	4.91±0.13 ***	5.07±0.43*	0.8±0.04 *	6.29±0.61*	1.09±0.01*

Values are expressed as Mean ± SEM. *, **, ***, ns - Mean values are significantly different when compared with lithiatic control mean values at P <0.05, P<0.01, P<0.001 and non-significant respectively

Effect of AEPV on Urine Volume and Urine biochemical parameters on 28th day was

1. Urinary volume in Lithiatic group is decreased, positive control group is increased. Urinary volume in AEPV in high dose is almost equal to the low dose.

2. Calcium, Oxalate, Phosphate level in Lithiatic group is increased, positive control and the extracts of AEPV in low dose and high dose may be decreased.

3. Magnesium level in Lithiatic group is decreased, positive control and the extracts of AEPV in low dose and high may be increased.

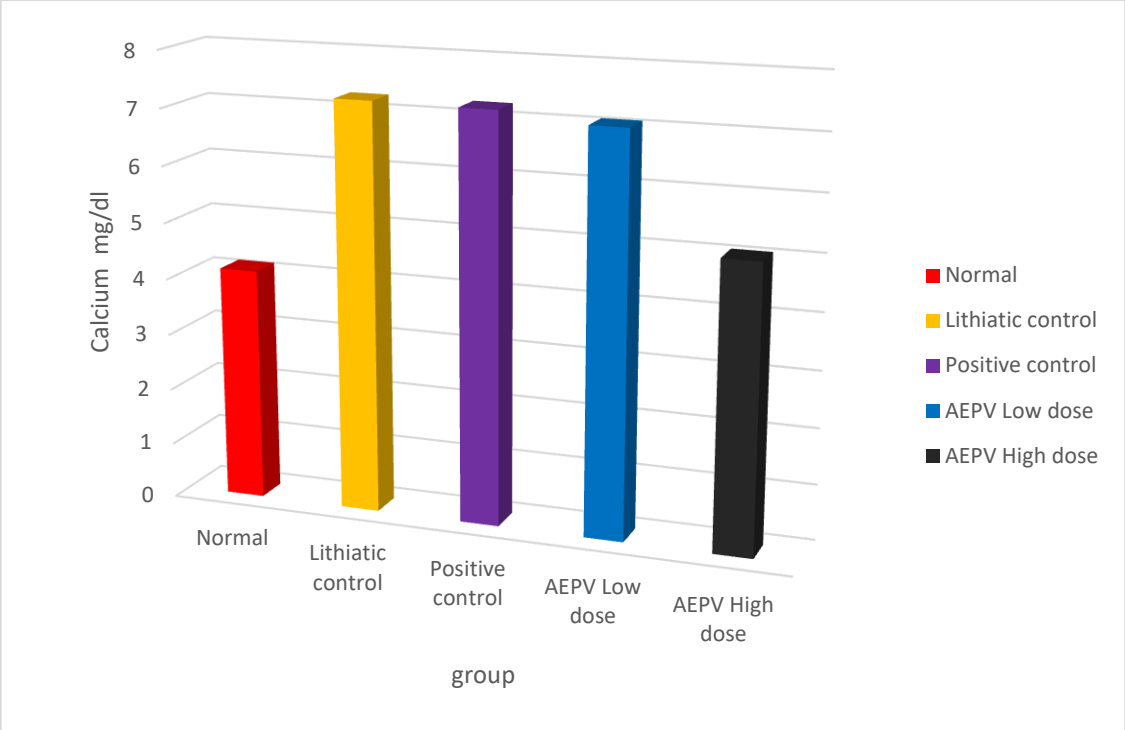


Figure No: 26. The calcium excretion in rats urine on day 28

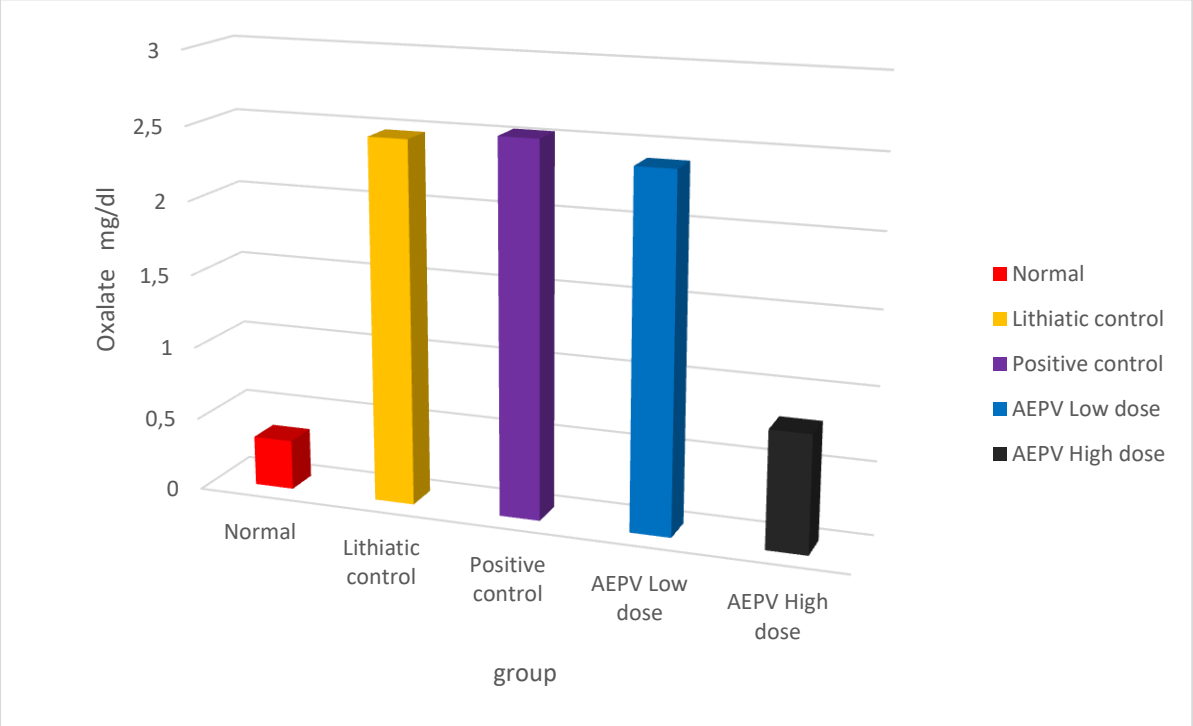


Figure No: 27. The oxalate excretion in rats urine on day 28

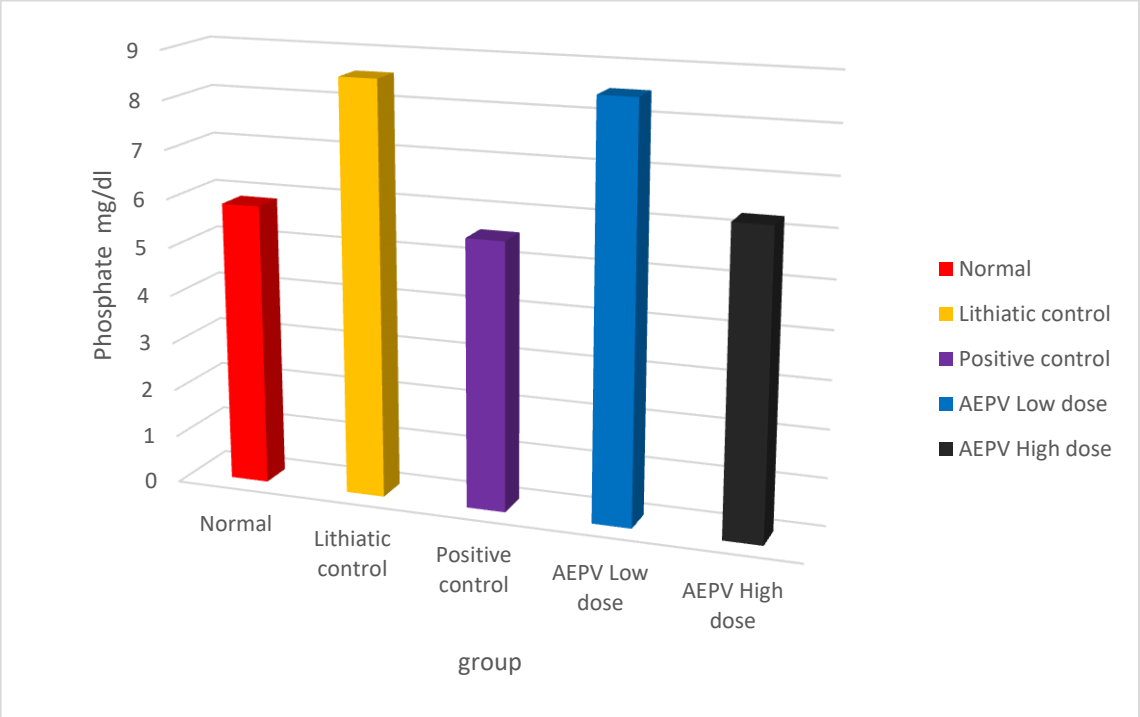


Figure No: 28.The phosphate excretion in rats urine on day 28

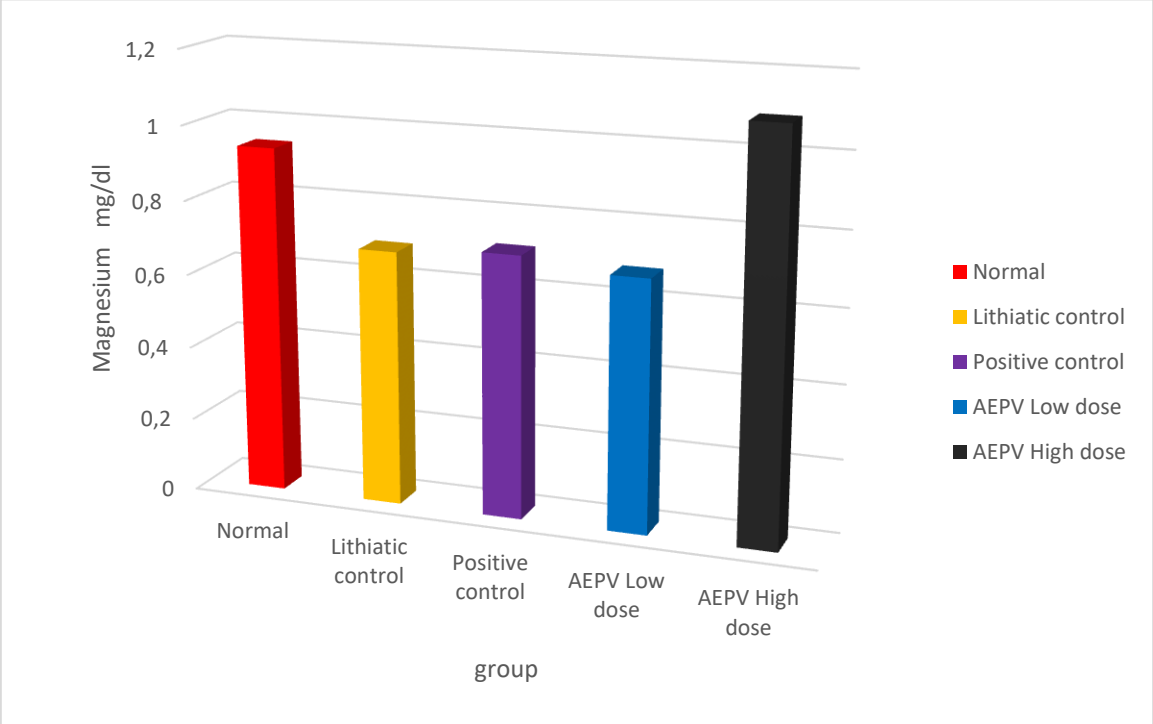


Figure No: 29. The Magnesium excretion in rats urine on day 28

Effect of AEPV on serum biochemical parameters on 28th day

Table No: 13. Effect of AEPV on serum biochemical parameters on 28th day

GROUP	SERUM ANALYSIS (mg/dl)		
	Creatinine	**Urea**	**BUN (Blood urea nitrogen)**
Normal	0.54±0.02	2.49±0.19	40.13±0.71
Lithiatic control (un treated)	0.99±0.06	4.11±0.25	61.60±0.66
Positive control (cystone treated)	0.39±0.002*	1.80±0.13*	37.41±0.78*
AEPV Low dose(200mg/kg)	0.75±0.05**	2.08±0.026*	42.70±0.94*
AEPV high dose(400mg/kg)	0.73±0.08**	1.04±0.16*	39.10±0.98*

Values are expressed as Mean ± SEM. *, **, ***, ns - Mean values are significantly different when compared with lithiatic control mean values at P <0.05, P<0.01, P<0.001 and non-significant respectively

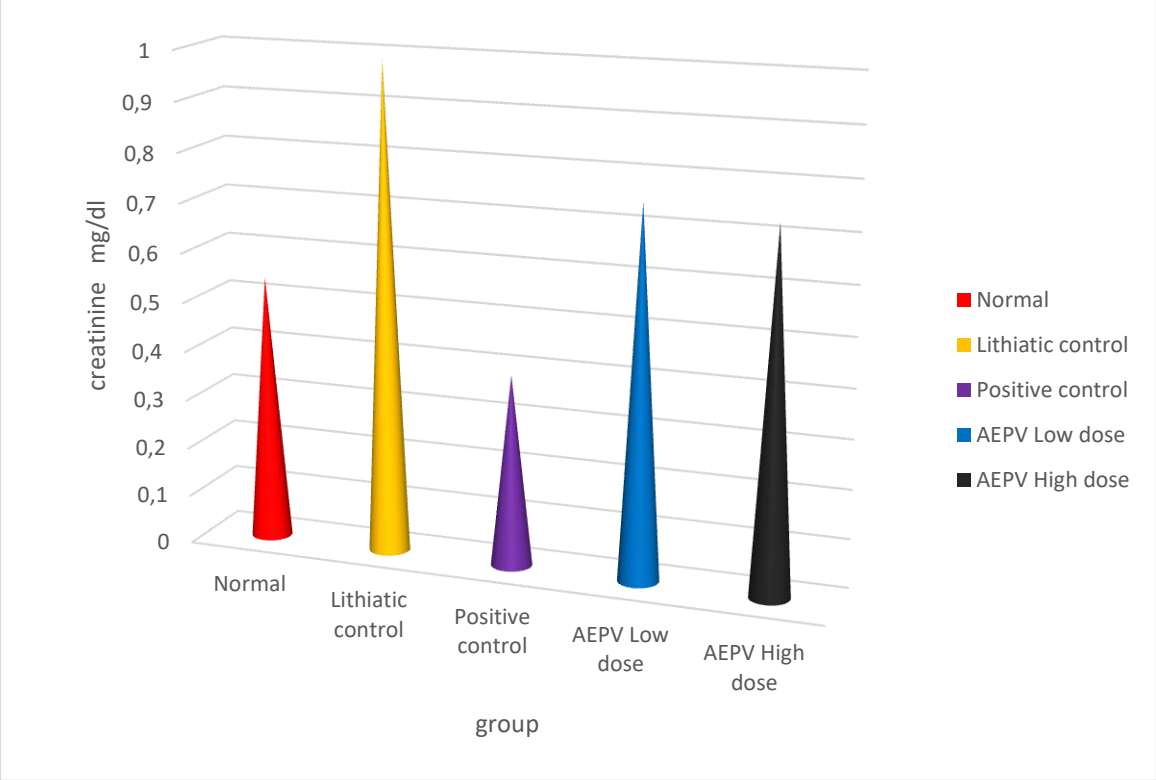


Figure No: 30. The creatinine excretion in rats blood on day 28

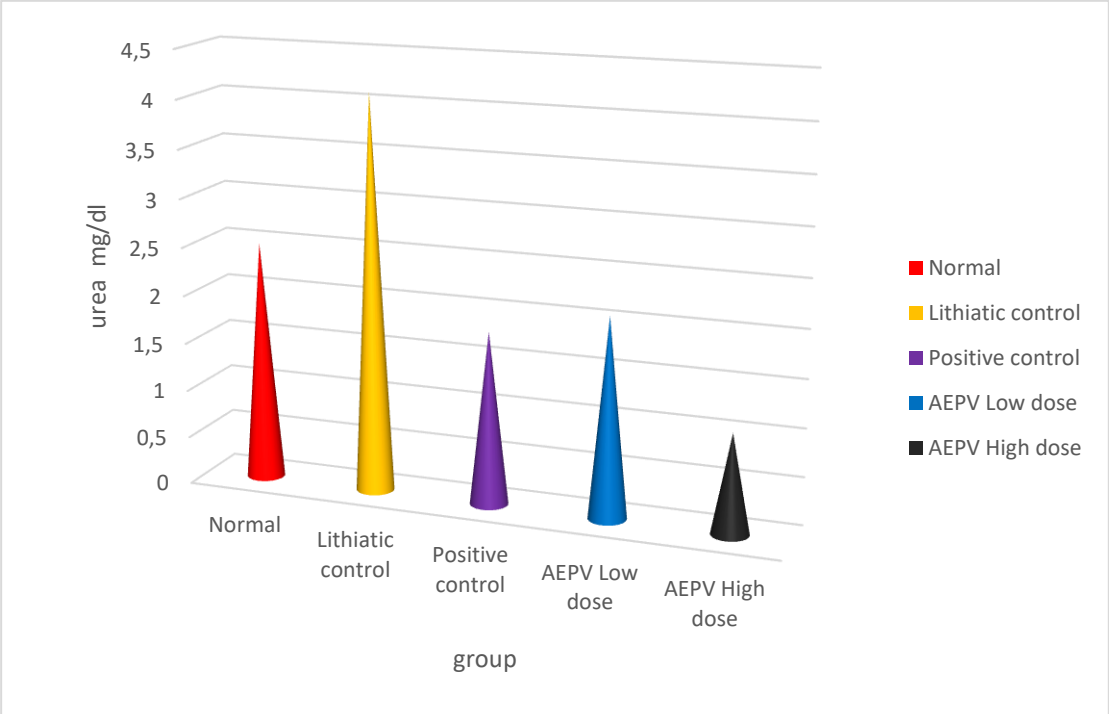


Figure No: 31. The urea excretion in rats blood on day 28

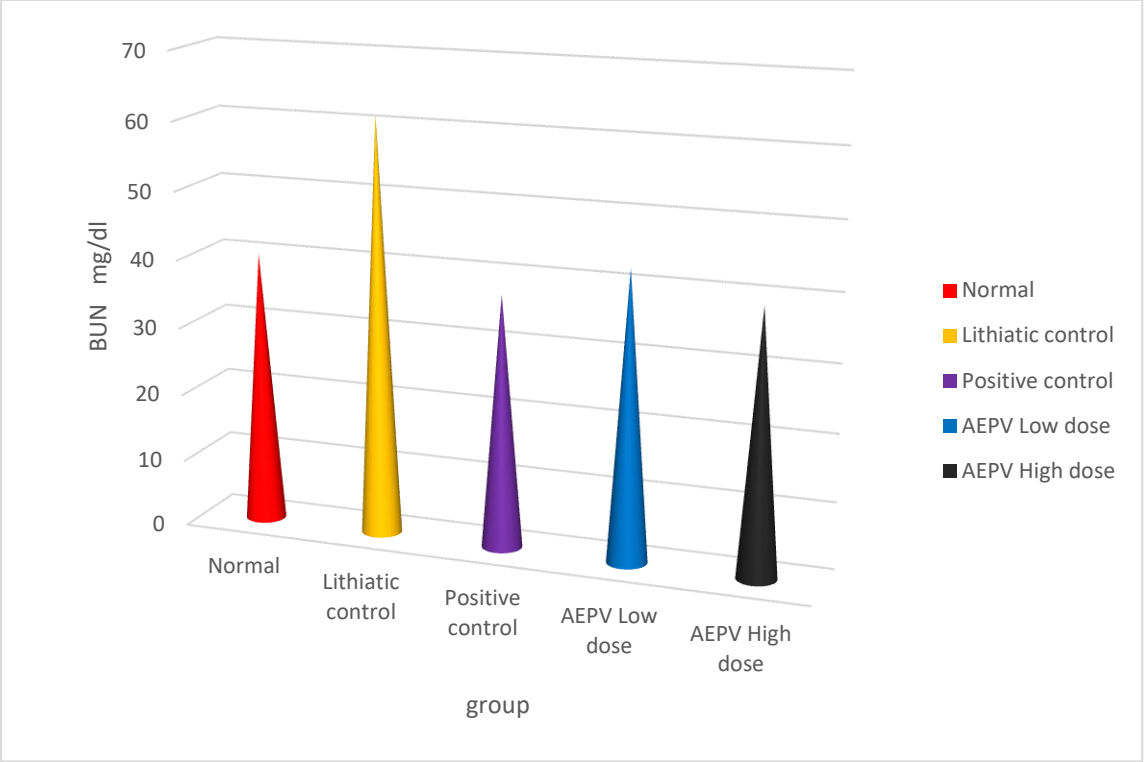


Figure No: 32. The BUN excretion in rats blood on day 28

Effect of EEPV on Urine Volume and Urine biochemical parameters on 28th day

Table No: 14 Effect of EEPV on Urine Volume and Urine biochemical parameters on 28th day

Group	URINE ANALYSIS				
	Urinary volume (ml/24 h)	**Calcium (mg/dl)**	**Oxalate (mg/dl)**	**phosphate (mg/dl)**	**Magnesium (mg/dl)**
Normal	7.89±0.12	4.40±0.59	0.34±0.013	5.83±0.39	0.96±0.59
Lithiatic control (un treated)	4.82±0.17	7.98±0.64	3.07±0.02	8.85±0.61	0.51±0.64
Positive control (cystone treated)	6.8±0.04	6.55±0.32**	1.58±0.04**	6.9±0.59**	1.31±0.32*
EEPV Low dose(200mg/kg)	7.23±0.11	6.15±0.51**	1.78±0.03**	7.83±0.56**	1.29±0.51*
EEPV high dose (400mg/kg)	7.21±0.20	6.13±0.46**	1.68±0.03*	7.31±0.217**	1.54±0.46*

Values are expressed as Mean ± SEM. *, **, ***, ns - Mean values are significantly different when compared with lithiatic control mean values at P <0.05, P<0.01, P<0.001 and non-significant respectively

Effect of EEPV on Urine Volume and Urine biochemical parameters on 28th day was

1. Urinary volume in Lithiatic group is decreased, positive control group is increased. Urinary volume in EEPV in high dose is almost equal to the low dose.

2. Calcium, Oxalate, Phosphate level in Lithiatic group is increased, positive control and the extracts of EEPV in low dose and high dose may be decreased.

3. Magnesium level in Lithiatic group is decreased, positive control and the extracts of EEPV in low dose and high may be increased.

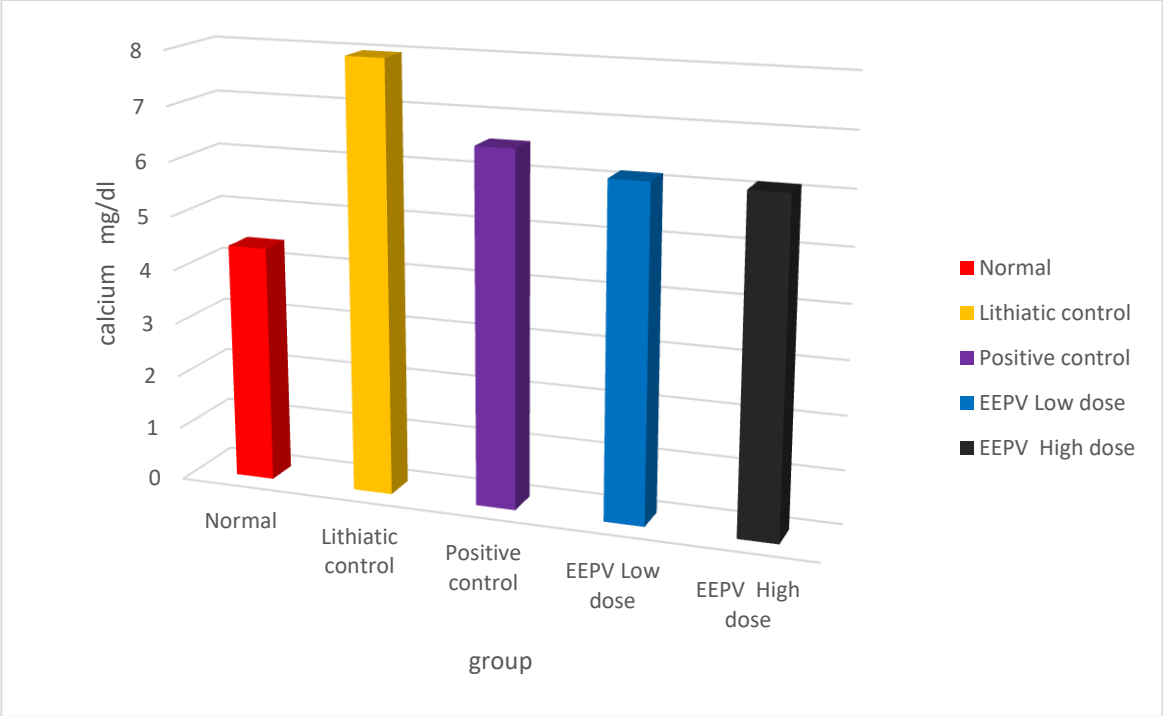


Figure No: 33. The calcium excretion in rats urine on day 28

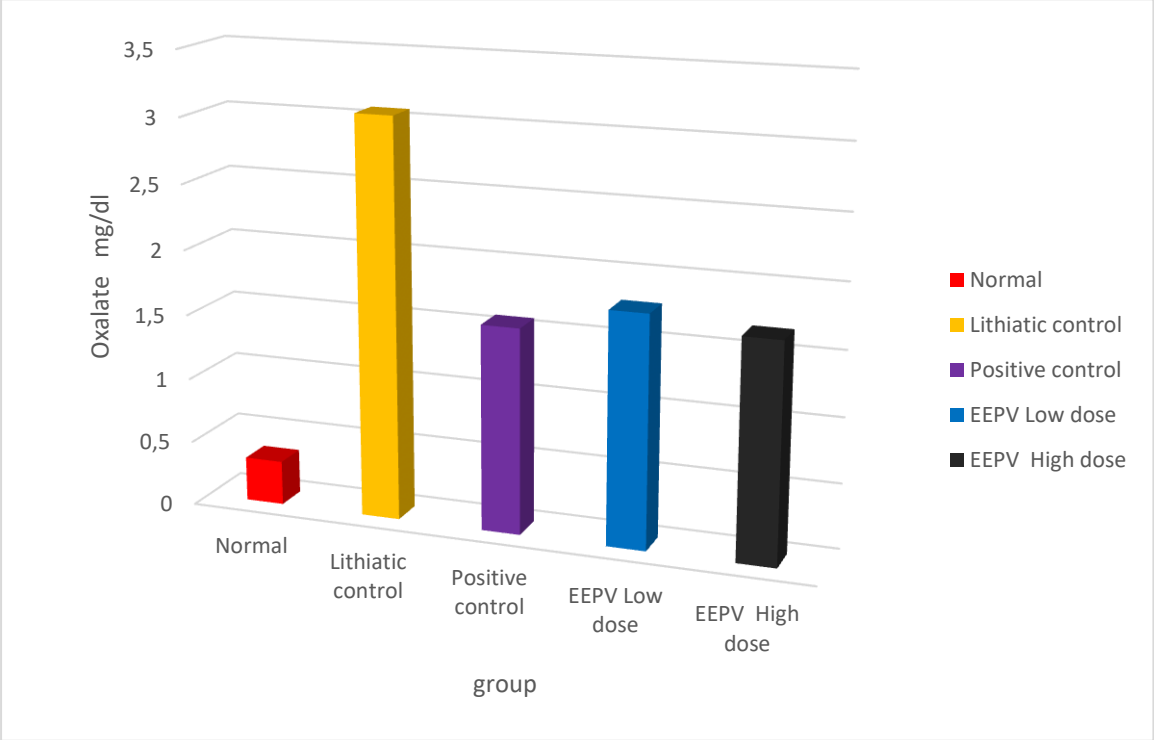


Figure No: 34. The Oxalate excretion in rats urine on day 28

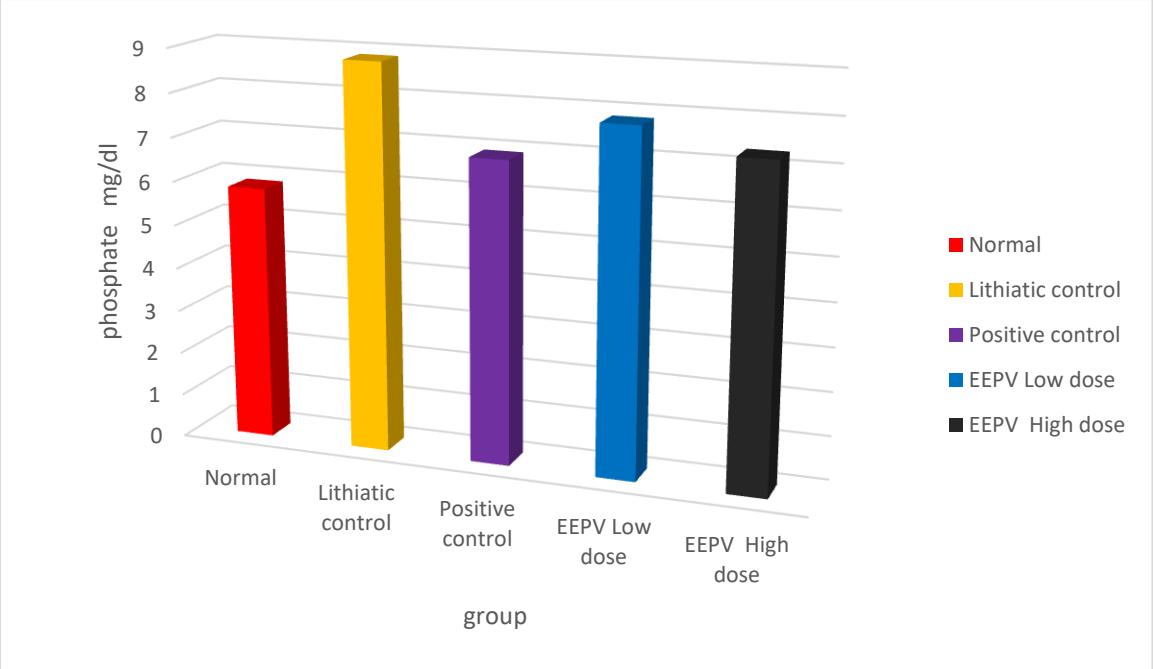


Figure No: 35. The Phosphate excretion in rats urine on day 28

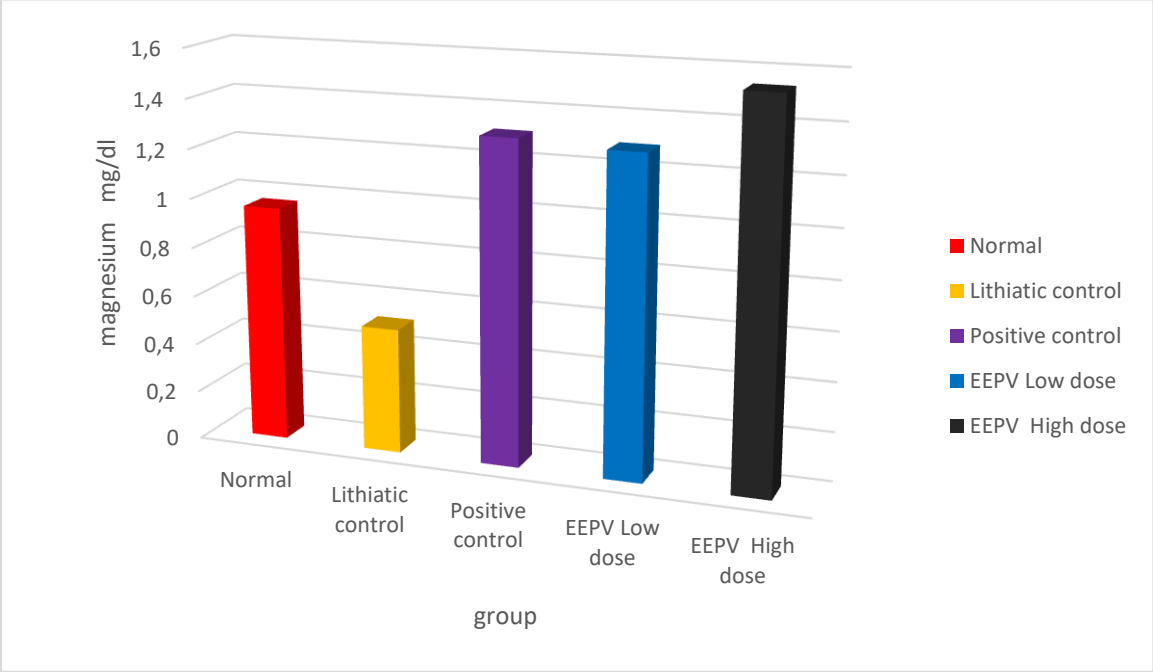


Figure No: 36. The Magnesium excretion in rats urine on day 28

Effect of EEPV on serum biochemical parameters on 28th day

Table No: 15. Effect of EEPV on serum biochemical parameters on 28th day

Group	Serum Analysis (mg/dl)		
	Creatinine	**Uric acid**	**BUN (Blood urea nitrogen)**
Normal	1.54±0.15	1.18±0.08	37.62±0.15
Lithiatic control (un treated)	2.31±0.05	2.56±0.03	41.20±1.45
Positive control (cystone treated)	1.82±0.05*	1.49±0.05*	26.70±1.48*
EEPV Low dose(200mg/kg)	1.85±0.04*	1.65±0.08*	35.21±1.22*
EEPV high dose (400mg/kg)	2.05±0.07*	2.10±0.16*	32.20±1.41*

Values are expressed as Mean ± SEM. *, **, ***, ns - Mean values are significantly different when compared with lithiatic control mean values at P <0.05, P<0.01, P<0.001 and non-significant respectively

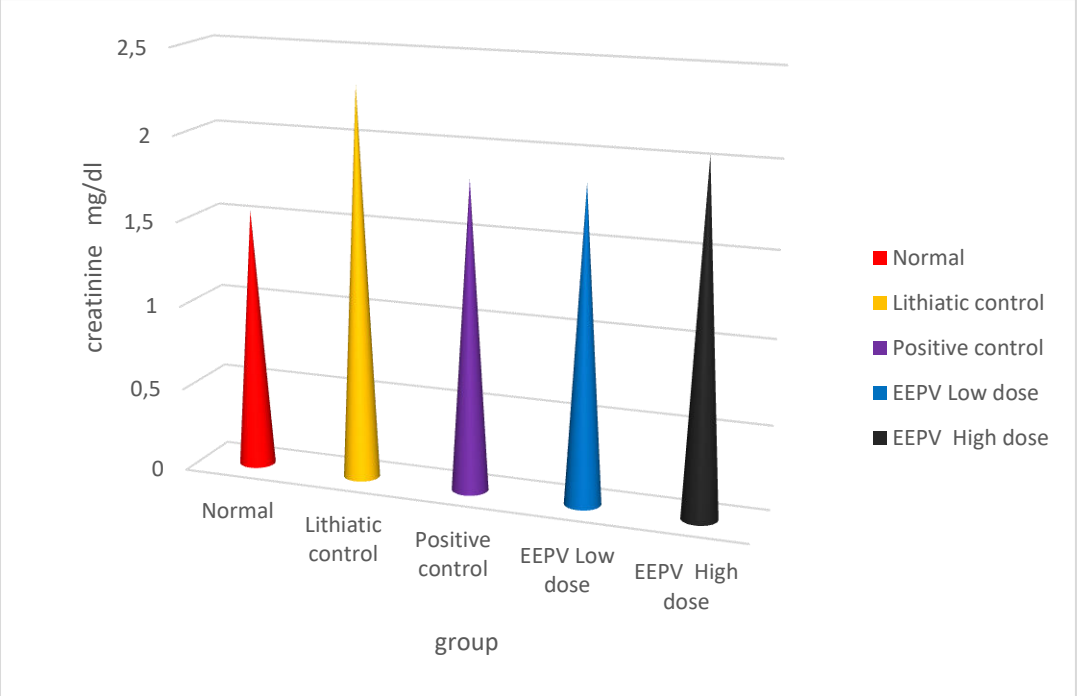


Figure No: 37. The Creatinine excretion in rats blood on day 28

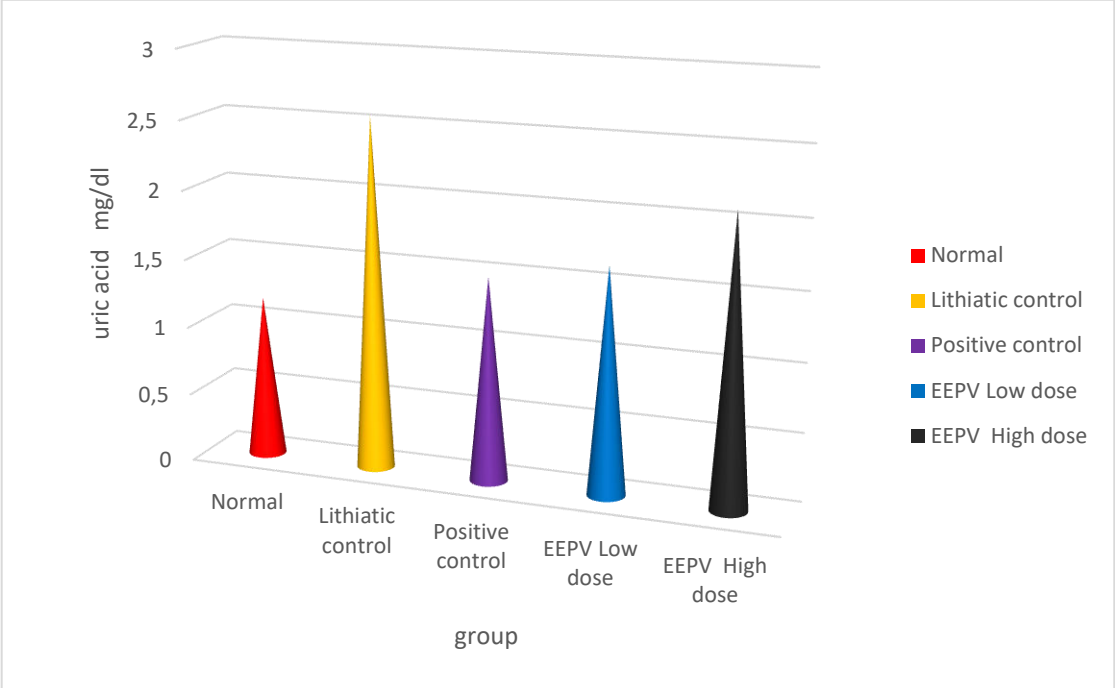


Figure No: 38. The uric acid excretion in rats blood on day 28

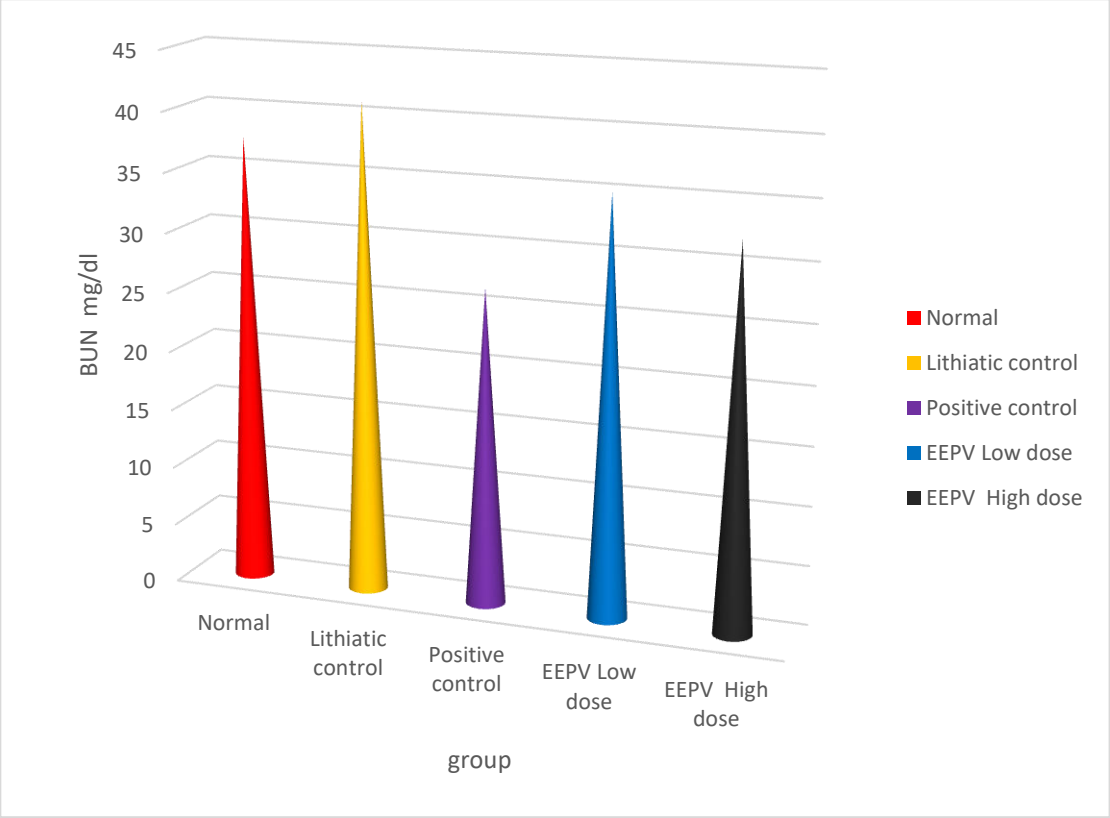
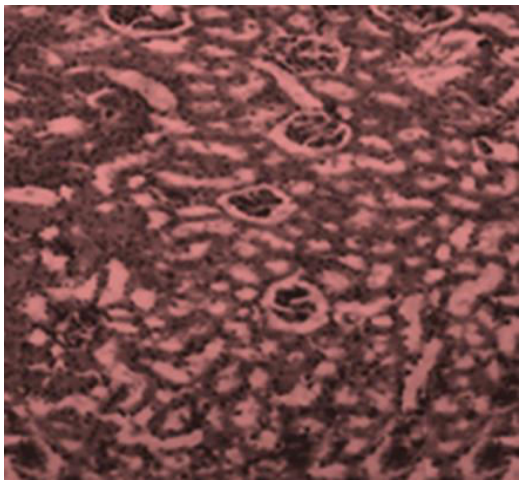


Figure No: 39. The BUN excretion in rats blood on day 28

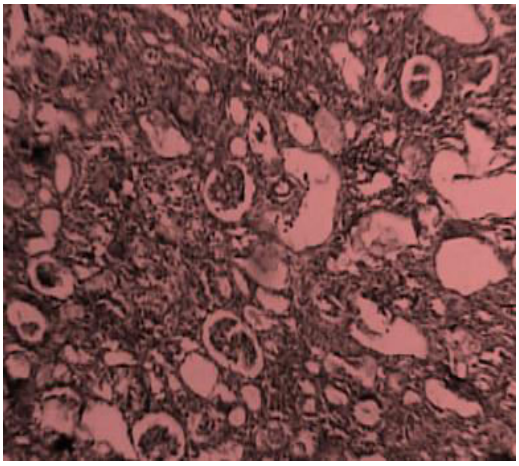
6. **HISTOPATHOLOGYSTUDIES:**

Histopathological findings of kidney under a light microscope (×40)

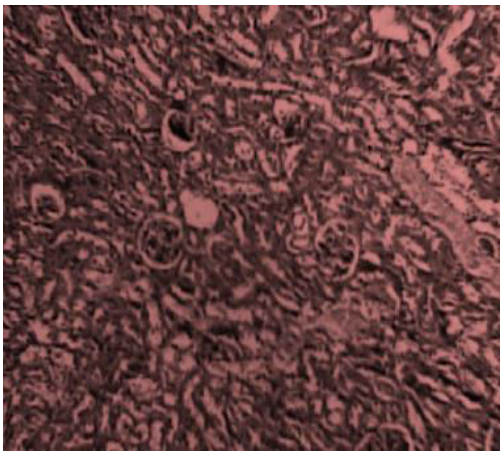
PREVENTIVE STUDY



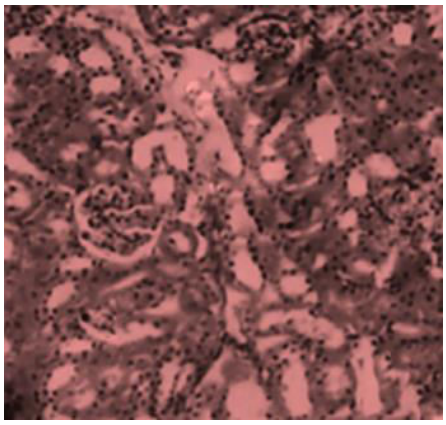
A. Normal



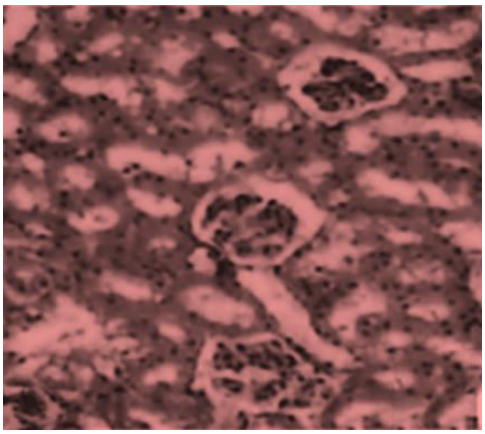
B. Lithiatic control



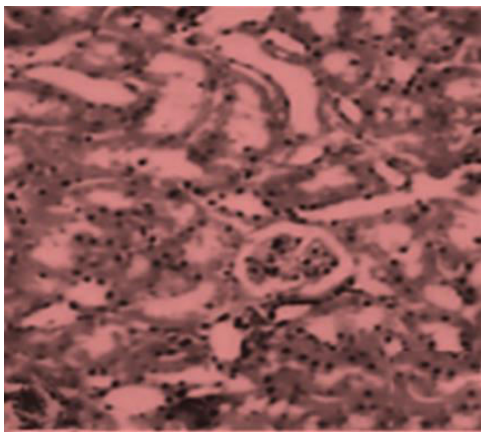
C. Low dose (AEPV 200 mg/kg)



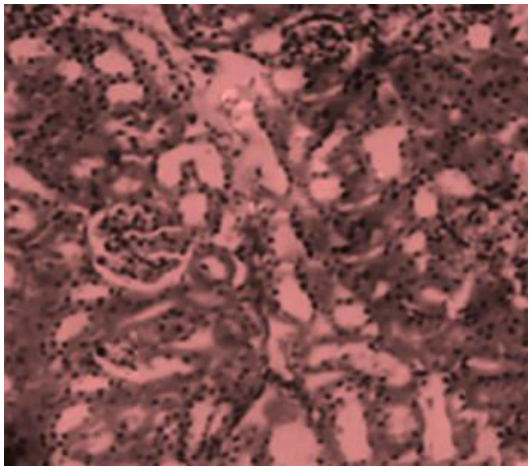
D. High dose (AEPV 400 mg/kg)



E. Low dose (EEPV 200 mg/kg)



F. High dose (EEPV 400 mg/kg)



G. Standard (Cystone)

Figure No: 40 Histopathology of kidney (preventive study)

A. Normal: Renal parenchyma with normal tubules, and glomeruli

B. Lithiatic control: Oxalate renal stone, tubular dilation and renal tubular damage, severe damage to the medulla, glomeruli, tubules.

C. Low dose (AEPV 200 mg/kg): Glomerular atrophy and tubular dilation.

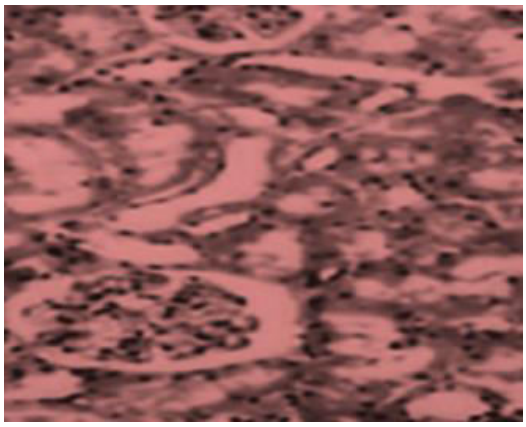
D. High dose (AEPV 400 mg/kg): Regenerated to normal glomerular structure.

E. Low dose (EEPV 200 mg/kg): Renal tubular dilation and glomerular atrophy.

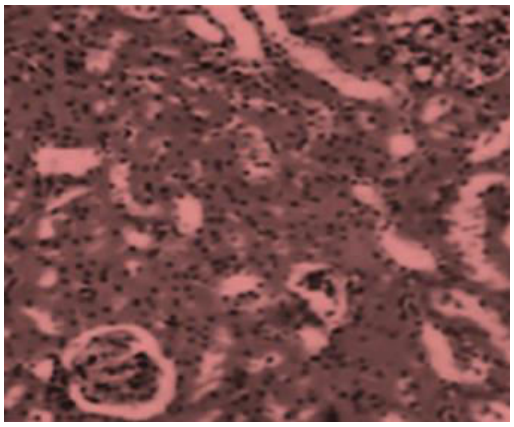
F. High dose (EEPV 400 mg/kg): Regenerated to normal glomerular structure

G. Standard (Cystone): mild vacuolar degeneration of renal tubular epithelial cells.

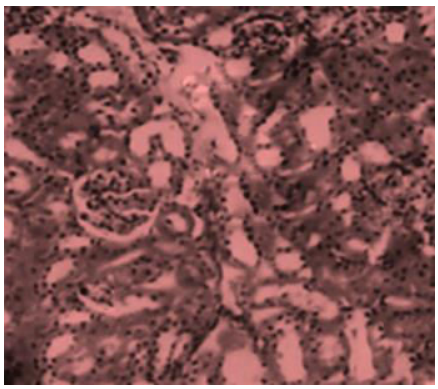
CURATIVE STUDY:



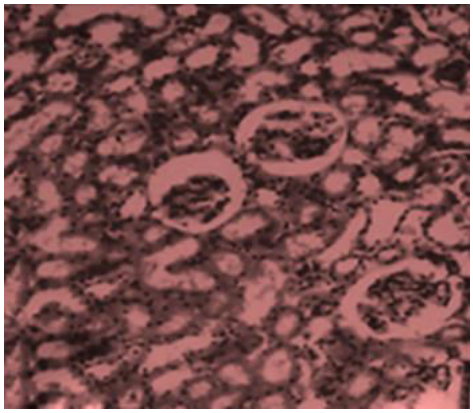
A. Low dose (AEPV 200 mg/kg)



B. High dose (AEPV 400 mg/kg)



C. Low dose (EEPV 200 mg/kg)



D. High dose (EEPV 400 mg/kg)

Figure No: 41. Histopathological study of kidney (Curative study)

A. Low dose (AEPV 200 mg/kg): presence of CaOx crystals in the lumen of dilated renal tubules.

B. High dose (AEPV 400 mg/kg): mild vacuolar degeneration of renal tubular epithelial cells

C. Low dose (EEPV 200 mg/kg): Renal tubular dilation and glomerular atrophy.

D. High dose (EEPV 400 mg/kg): Regenerated to normal glomerular structure.

DISCUSSION

Formation of kidney stones is a complex process and involves a series of biological events that are most likely triggered by genetic susceptibility together with dietary factors and lifestyle changes. Several in vivo animal models have been developed to investigate the mechanisms involved in the formation of urinary stones. However, rat model has been widely used for the study of CaOx deposition in the kidneys, a process that mimics the etiology of kidney stone formation in humans. Consequently kidney stones formation was induced in wistar albino rats by ethylene glycol and ammonium chloride in drinking water model in the present study.

EXTRACTION AND PRELIMINARY PHYTOCHEMICAL STUDIES:

The percentage yield of ethanolic and aqueous extract of *Phaseolus vulgaris linn* was found to be 8.24 % w/w and 6.32 % w/w respectively.

The ethanolic extract (EEPV) was dark brown in colour with a thick viscous consistency. The aqueous extract (AEPV) also has a thick viscous consistency and pale yellow in colour. Preliminary Phytochemical screening of EEPV and EEPV revealed the presence of Alkaloids, Carbohydrates, Proteins & amino acids, Steroids, Phenols, Tannins, Flavonoids, Terpenoids and Glycosides while the aqueous extract alone possesses saponins. **(Table No:1)**

These phytochemicals may be responsible for the anticrystallization and antioxidant activities. Plant flavonoids are reported to possess antiurolithiatic activity through its antioxidant property. Several reports suggest that saponins are having antiurolithiatic activity through its diuretic and disaggregating the suspension of mucoproteins. [9]

The results of TLC separation of the extracts with the mobile phase Chloroform: Methanol: Distilled water: Toluene - 8:1:0.5:0.5 followed by spraying with Iodine showed 2 spots for aqueous extracts and 4 spots for ethanolic extract (Fig No:6). As the mobile phase used is specific for saponins and terpenes, [74] the isolated compounds may be the same. The Rf value one of the compounds (0.86) separated with methanolic extract of Phaseolus vulgaris seeds using the same mobile phase almost coincides with one of the spots of our aqueous extract (0.83) and one spot in ethanolic extract (0.88). The other spots in the ethanolic extracts have Rf values almost similar to that of methanolic extracts of Phaseolus

lunatus methanolic extracts. Therefore the spots visualised in the extracts indicate the presence of either saponins or terpenes in aqueous extract and only terpenoids in ethanolic extract. Therefore the TLC can be the identifying fingerprinting for these extracts in future. **(Table No:2)**

Determination of Phytoconstituents of extracts:

Total Flavonoid Content of AEPV was found to be 8.81 mg Quercetin Equivalents / g plant Extract.

Total Flavonoid Content of EEPV was found to be 9.18 mg Quercetin Equivalents / g plant Extract **(Table No: 3)**

Total Phenolic Content of AEPV was found to be 30.10 mg Gallic acid Equivalents / g plant Extract.

Total Phenolic Content of EEPV was found to be 42.22 mg Gallic acid Equivalents / g plant Extract **(Table No: 4)**

Total Flavonoid Content of EEPV (9.18 mg) was found to be more compared with the AEPV (8.81 mg).

Total Phenolic Content of EEPV (42.22 mg) was found to be more compared with AEPV (30.10 mg)

***In vitro* antioxidant studies on the extracts:**

The anti oxidant activity of AEPV and EEPV was assessed by three methods namely nitric oxide scavenging assay, Reducing power assay and in vitro lipid peroxidation assay.

A lower value IC50 observed for aqueous extract of PV (195 µg/ml) in Nitric oxide scavenging assay compared to that of the ethanolic extract (651 µg/ml) indicates the antioxidant potential possessed by the aqueous extract is greater than that of its ethanolic counterpart. However the antioxidant efficacy of the aqueous extract is lower than that of the standard Ascorbic Acid (IC50-98.26 µg/ml). **(Table No: 5)**

Reducing power assay is also performed to evaluate the antioxidant activity of the extracts. In this method, higher absorbance values obtained with the reaction mixtures denote higher reductive potential of the testing substance. Reducing power is considered a significant index of the antioxidant activity as the molecules with good reducing power are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes and thus as primary and secondary antioxidants.[72] The observations in the present model also

demonstrate the greater antioxidant potential of the aqueous extract compared to the ethanolic extract. As the concentration of the testing substance increases the reducing power also was found to increase. **(Table No: 6)**

Lipid peroxidation involves the formation and propagation of lipid radicals, which eventually destroy membrane lipids. Oxidative stress in cells and tissues can be best monitored by its lipid peroxidation assay. The TBARS formation assay was used to assess the inhibition of Fe2+-induced lipid peroxidation by the extract. In Lipid peroxidation assay the percentage of inhibition and IC50 value are calculated. In lipid peroxidation inhibition assay, the plant extract inhibits the FeSO4 induced lipid peroxidation in egg yolk, which is the net result of iron-mediated hydroxyl radicals. This can be achieved either by scavenging the hydroxyl radicals or by chelating the iron ions, which is responsible for initiation of Fenton's reaction.[73]

IC50 value of Standard Ascorbic Acid in lipid peroxidation assay was found to be 171.66 µg/ml. Both extracts showed a very good concentration-dependent inhibition of lipid peroxidation and almost equal activity (IC50 of aqueous and ethanolic extracts -732.33 µg/ml, 700.66 µg/ml respectively). **(Table No: 7).**

Impaired protection against oxidative stress resulted in calcium oxalate crystal deposition in kidney and treatment with antioxidants prevent this CaOx deposition. [25] Also Earlier studies report that membrane lipid peroxidation caused by high levels of calcium oxalate crystals induces cellular injury. [76] Possession of antioxidant activity implies that the extracts will prevent the calcium oxalate deposition in the kidney.[77]

The polyphenols, tannins, content of the extracts have been reported to have metal chelating and hydroxyl radical scavenging properties. [9]

Anti urolithiatic activity:

Ethylene glycol and ammonium chloride are always used as urolithiasis induction agents because they induce Calcium oxalate crystalluria without severe renal damage in rats and they mimic the etiology of stone formation in human. [33]

Ammonium chloride causes acidification of urine consequently decreasing the citrate excretion in urine. This, in turn, increases the deposition of renal Calcium oxalate crystals and accelerates the Lithiasis. Induction of Calcium oxalate type of urolithiasis by Ethylene

glycol is a well-validated and clinically relevant model. Ethylene glycol is metabolized to form acidic metabolites such as oxalic acid, benzoic acid, formic acid, and hippuric acid, which causes metabolic acidosis. Subsequently, this acidic condition favours Calcium oxalate nucleation followed by growth, aggregation and crystallization, then finally retention at the renal tissue to cause renal mitochondrial toxicity similar to clinical Calcium oxalate renal calculi. [19]

On administration of Ethylene glycol and Ammonium chloride in drinking water for 15 days, a significant increase in the deposition of calcium and oxalate levels in the kidney were observed in both the preventive-control (Lithiatic) and curative-control (Lithiatic) groups when compared to the normal.

In the preventive regimen, animals treated with cystone and AEPV and EEPV at doses 200 and 400 mg/ kg, a significant decrease in calcium ($p<0.05$) and oxalate levels in urine was observed when compared to the preventive-control group (Lithiatic). In the curative regimen, animals treated with cystone and AEPV and EEPV also showed a significant($p<0.05$, $p<0.01$) decrease in calcium and oxalate deposition in the kidney when compared to curative-control group (Lithiatic). These effects were found to be dose-dependent.

Urine volume also played a major role in the Calcium oxalate stone formation. In this study, a decrease in urine output was observed in both preventive-control group (Lithiatic) and curative-control group (Lithiatic), indicating an obstruction in the urinary flow due to the presence of the Calcium oxalate stones. An increase in the urinary output was observed on treatment with EEPV and AEPV, indicating its diuretic action. Further, it dilutes the urinary electrolytes concentration and might decrease the chance of stone development. [31]

An increase in urinary phosphate excretion was observed in both the preventive-control (Lithiatic) and curative-control (Lithiatic) groups when compared to the normal. Elevated urinary phosphate excretion along with oxalate induced stress appears to provide a suitable environment for stone formation by forming calcium phosphate crystals, which causes Calcium oxalate deposition. Treatment with standard (cystone) and the plant extract treated (EEPV and AEPV) lowered the excretion of phosphate and reduced the risk of stone formation.

Supersaturation is the step that occurs due to the presence of substances producing the kidney stones in high concentration in the urine following the decrement of urine volume and the concentration of chemicals that inhibit stone formation. [29] In our study the magnesium level in urine showed a significant decrease in urolithiatc group upon administration of ethylene glycol when compared to control group, the increase in urine magnesium level was recovered in animals that treated by standard (cystone) and the plant extract treated (EEPV and AEPV).

In urolithiasis, there is a decrease in the glomerular filtration due to the obstruction of urine flow by stones in urinary system. This causes impairment of renal function resulting in decreased excretion of waste products, particularly nitrogenous substances such as Urea, Creatinine, and BUN with concurrent accumulation in blood. In the present study administration of Ethylene glycol with Ammonium chloride showed a significant elevation in serum Creatinine, Urea and BUN excretion was observed in both the the preventive-control (Lithiatic) and curative-control (Lithiatic) groups when compared to the normal group which indicates the marked renal damage. It was accompanied by the decreased glomerular filtration rate (GFR) due to obstruction of stones in the Bowman's capsule. However, treatment of rats with standard (cystone) and the plant extract treated (AEPV and EEPV) show decreased serum Creatinine, Urea and BUN through improved glomerular filtration rate.

An in vitro study suggests that phytic acid process anti-crystallization property by its chelating nature with the calcium. *Phaseolus vulgaris* beans are rich in phytic acid, which combines with calcium to form calcium-phytate complex that reduces the availability of calcium for stone formation by reducing its intestinal absorption. [9]

Histopathological study:

These results supported by our histopathological study that showed extensive vacuolar degeneration of renal tubular epithelium with presence of Calcium oxalate crystals in the lumen of dilated renal tubules in ethylene glycol group while the treatment of rats with standard (cystone) plant extracts(AEPV and EEPV) reduced histopathological alterations

The standard treated groups showed normal histology of the kidney, and shows normal glomeruli, slight oedema of the tubular cells. The AEPV & EEPV treated animals also showed the recovery

Kidneys of normal rats showed normal renal glomeruli and tubules with no evidence of tubular degeneration, dilatation or inflammatory reaction. Meanwhile, characteristic histopathological alterations were demonstrated in the Lithiatic control group represented by extensive vacuolar degeneration of renal tubular epithelium and presence of Calcium oxalate crystals in the lumen of renal tubules which are greatly dilated. In preventive study marked improvement of the histopathological alterations was demonstrated in Cystone and plant extract treated (AEPV and EEPV) group manifested by absence of inflammatory reaction, mild tubular degeneration and decreased number of renal tubules containing calculi. On the other hand, Curative treatment with plant extract treated (AEPV and EEPV) group showed the mild improvement represented by vacuolar degeneration of renal tubules and presence of Calcium oxalate crystals in the lumina of some renal tubules.

The Antioxidant activity as well as the antiurolithiatic activity of the extracts can be attributed to the presence of the phytoconstituents flavonoids, polyphenols, tannins, and saponins because plant flavonoids are reported to possess antiurolithiatic activity through its antioxidant property.[77] The findings of other studies reveal that saponins have antiurolithiatic activity through its diuretic action and their ability in disaggregating the suspension of mucoproteins .

The mechanism of the antiurolithiatic activity of the aqueous and ethanolic extracts of Phaseolus vulgaris may be through their potential of lowering stone forming constituents and by their antioxidant property. In future this study can be extended to perform bioactivity guided fractionation and to isolate the active principles responsible for the antiurolithiatic activity of phaseolus Vulgaris & and to probe the mechanisms of action.

CONCLUSIONS

The results of the present investigation on the evaluation of antiurolithiatic activity of aqueous and ethanolic extracts in rat models of preventive and curative lithiasis have led to the following conclusions.

- In preventive and curative studies of AEPV and EEPV showed significant reduction in calcium, oxalate and phosphate levels in urine & an increase in the urinary magnesium and a restoration of normal urine volume. Serum levels of creatinine, uric acid and BUN were also brought down to normal values by the extracts. Histopathological observations also confirm the same. These findings indicate the potential of the extracts in inhibiting kidney stone formation as well as a lithotripsic action on the formed renal stones.
- Aqueous extract is more potent than the ethanolic extract in the urolithiasis rat models.
- The promising antioxidant activity of the extracts as revealed by in vitro nitric oxide scavenging assay, reducing power assay and in vitro lipid peroxidation study would have contributed to the antiurolithiatic activity of the extracts
- Flavonoids, Polyphenols and saponins present in the extracts may be responsible of the antioxidant and antilithiatic activity of the extracts

BIBLIOGRAPHY

1. Khaling Mikawlrawng, Suresh Kumar, Vandana. Current scenario of Urolithiasis and the use of medicinal plants as antiurolithiasis and the use of medicinal plants as antiurolithiatic agents in Manipur (north east india): A Review International Journal of Herbal Medicine. 2014; 2(1): 1-12.

2. Nalini H S, Manickavasakam k, Thomas Walter M. Prevalence and risk factors of kidney stone. Global Journal for Research Analysis. 2016; 5(3): 183-187.

3. Tilahun Alelign and Beyene Petros. Kidney stone diseases: An update on current concepts. Hindawi Advances in Urology. Volume 2018; Article ID 3068365: 12 pages.

4. Apurba Marak, Shantibala K, Th. Achouba Sing, RK Narendra Singh, L Shanti Kumar Singh. Urolithiasis: prevalence and related factors in a rural area of Manipur. International Journal of Medical Science and Public Health. 2013; 2(4): 956-959.

5. Bahuguna Y M, Rawat M S M, Juyal, V, Gnanarajang. Antilithiatic effect of grains of *eleusine coracana*, Saudi Pharmaceutical Journal. 2009; 17(2): 155-158.

6. Andrew P E. Physiopathology and etiology of stone formation in the kidney and the urinary tract, Pediatric Nephrology. 2010; 25: 831-841.

7. Ankur C, Amarchand P, Aadarsh C, Deepa I R S, Patil UK. Potential of medicinal plants in kidney, gall and urinary stones, International Journal of Drug Development & Research. 2010; 2(2).

8. Atmani F, Slimani Y, Mimouni M, and Hacht B. Prophylaxis of calcium oxalate stones by Herniaria hirsute on experimentally induced nephrolithiasis in rats. BJU International. 2003; 92: 137-140.

9. Sree Lakshmi, Namburu Sujatha, Dodoala, Bharathi Koganti K, S.Prasad. Antiurolithiatic activity of phaseolus vulgaris seeds against ethylene glycol-induced renal calculi in wistar rats. International Journal of Green Pharmacy. Oct-Dec 2017; 11(4): 281.

10. B D Chaurasia's . Human Anatomy Regional and applied /Dissection and clinical /lower limb Abdomen and Pelvis. Seventh edition; vol-2: 2017 page no: 341-343.
11. Kidney-Structure, Anatomy and Function – www.online biology notes.com/ kidney-structure/ kidney-Anatomy/ kidney-Function.
12. Definition of Urolithiasis - Medicine Net. https:// www.medicinenet.com/ main/ art.
13. Harsh Mohan. Text Book of Pathology. Seventh edition; page no: 672-674.
14. Brunner and Suddarth's. Text Book of Medical-Surgical Nursing. 13th edition; vol-2: page no: 1591-1595.
15. Lewis's Medical –Surgical Nursing. Second edition; vol-2: page no: 1129-1133.
16. Kidney stones - Symptoms and Causes – mayoclinic .https:// www.mayoclinic.org/ diseases-conditions/ kidney-stones/ symptoms-causes/ syc-20355755 and h1=en-IN.
17. Maso T, Sujihata. The role of urinary kidney stone inhibitors and promoters. International Journal of Urology. 2008; 15: 115-120.
18. Doddametikurre Ramegowada, Chandra, Shekhar Biyani, Antony J B, Jon J, Cartledge. The role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones. . International Journal of Urology. 2007; 5: 126-136.
19. Ureter Stones: Symptoms types causes treatment, diagnosis, prevention https:// www.epainassist.com/pelvic-pain /urethera / ureter -stones.
20. Christian Turk, Ales Petrik, Kemal Sarica, Christian Seitz, Andreas Skolarikos, Michael Straub, Thomas Knoll. EAU guidelines on diagnosis and conservative management of urolithiasis. European Urology. 2016; 69: 468-474.
21. Sandhya A, Sandhya Devi G, Sreedevi V, Deepika P, Hema Prasad M. 2010. Kidney Stone Disease: Etiology And Evaluation. International Journal of Applied Biology and Pharmaceutical Technology. 2010; 1(1): 175.
22. Andrew P E. Physiopathology and etiology of stone formation in the kidney and the urinary tract, Pediatric Nephrology. International Journal of Applied Biology and Pharmaceutical Technology. 2010; 25: 831-841.
23. kidney stone disease – Wikipedia. https://en.m.wwikipedia.org/wiki/ kidney-stone-disease.
24. Atef M, Al-Attar. Antilithiatic influence of *spirulina* on Ethylene Glycol-Induced Nephrolithiasis in Male rats, American Journal of Biochemistry and Biotechnology. 2010; 6(1): 25-31.

25. Sathish R, Jeyabalan G. Study of invitro Anti lithiatic effect of *Ipomoea batatas (L)* leaves and Tuberous roots. Asian Journal of Pharmaceutical and Clinical Research. 2018; 11(2): 427-431.
26. Abdullah H, AL Gamli, Abeer A, Salama A, Shayoub M, Elhassen, Zoahair M, Osman, EL-ERAKY, Azza Hassan. Evaluation of Anti Urolithiatic activity of *Phoenix dactyleferea* seeds extract in ethylene glycol induced Urolithiasis in rats. International Journal of Pharmacy and Pharmaceutical Research. 2017; 9(2): 7-20.
27. Chandra Shekar Tailor, Anju Goyal. Isolation of Phyto constituents and invitro Anti lithiatic by Titrimetric method, Antioxidant activity by 1, 1-Diphenyl-2-Picryl hydrazyl Scavenging assay method of Alcoholic roots and rhizomes extract of *Hedychium Coronarium J.Koenig* plant species. Asian Journal of Pharmaceutical and Clinical Research. 2015; 8(4): 225-229.
28. Kamil M, AL- Jobori, Gwan J. Abdul-Kadhum. Plant Formulation extract prevents Sodium Oxalate induced in the kidney. International Journal of Medical Science and Clinical Inventions. 2015; 2(7): 1167-1178.
29. Saranya R, Geetha N. Inhibition of Clacium Oxalate Crystallization invitro by the extract of Beet root (*Beta vulgaris L*). International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(2): 361-365.
30. Kumkum Agarwal, Ranjana, Varma *Ocimum gratissimum L.*: A Medicinal Plant with Promising Antiurolithiatic Activity. International Journal of Pharmaceutical Sciences and Drug Research 2014; 6(1): 78-81.
31. Naga Kishore R, Mangilai T, Anja Neyulu N, Abhinayani G and Sravya N. Investigation of anti urolithiatic activity of *Brassica Oleracea gongylodes* and *Desmostachya bipinnata* in experimentally induced Urolithiasis in animal models. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(6): 602-604.
32. Narendra Vyas and Ameeta Argal. Antiurolithiatic Activity of Extract and Oleanolic Acid Isolated from the Roots of *Lantana camara* on Zinc Disc Implantation Induced Urolithiasis. ISRN Pharmacology; Volume 2013: ArticleID951795, page no: 1-5.
33. Ajij Ahmedan, Abdul Waduda, Nasreen Jahana, Alia Bilala, Syeda Hajera. Efficacy of *Adiantum capillus veneris Linn* in chemically induced urolithiasis in rats. Journal of Ethnopharmacology . 2013; 146: 411–416.
34. Nirmaladevi R, Jayaraman Uthaya Chandrikka, Gurusamy Annadurai, Kalpana S, Shrinidhi Raj T. Evaluation of *Aerva Lanata* Flower extract for its Antilithiatic Potential

invitro and invivo. International Journal of Pharmacy and Pharmaceutical Science Research. 2013; 3(2): 67-71.

35. Kameshwaran S, Thenmozhi S, Vasuki K, Dhanalakshmi M, Dhanapal C. Antiuro lithiatic activity of aqueous and methanolic extracts of *Tecoma Stans* Flower in Rats. International Journal of Pharmaceutical and Biological Archives. 2013; 4(3): 446-450.
36. Baheti DG, Kadam SS. Antiurolithiatic activity of a Polyherbal Formulation against calcium oxalate induced Urolithiasis in rats. Journal of Advanced Pharmacy Education and Research. 2013; 3(1): 31-41.
37. Kachchhi NR, Parmar RK, Tirgar PR, Desai TR, Bhalodia PN. Evaluation of the Anti Urolithiatic activity of methanolic extract of *Celosia argentea* roots in rats. International Journal of Phyto Pharmacology. 2012; 3(3): 249-255.
38. Pareta SK. *In vitro* Calcium Oxalate Crystallization inhibition by *Achyranthes indicalinn* Hydroalcoholic extract, An approach to antilithiasis. International Journal of Pharma and Biosciences. 2011; 2(1): 432-437.
39. Manabu Moriyama T, Koudai Suga, Katsuhito Miyazawa, Tatsuro Tanaka, Masaya Hagashioka, Kumiko Noda, Michiko Oka, Mitsushi Tanaka and Koji Suzuki. Inhibitions of urinary oxidative stress and renal calcium level by an extract of *Quercus salicina Blume/ Quercus stenophylla Makino* in a rat calcium oxalate urolithiasis model. International Journal of Urology. 2009; 16: 397–400.
40. Louise Anin Anin Atchibri, Lêniféré Chantal Soro, Jean Jacques Diagou, Dago Gnakri. Effect of Aqueous Extract of *Phaseolus Vulgaris* on the Regulation of Glucose and Lipid Metabolism in Diabetic Rats. Journal of Biotechnology and Biochemistry .2017; 3(1): 43-49.
41. Salman Ahmed , Muhammad Mohtasheemul Hasan and Zafar Alam Mohammad . Inhibition of calcium oxalate crystals growth by *Macrotyloma uniflorum (Lam.) Verdc*, *Phaseolus lunatus Linn, and Phaseolus vulgaris Linn*: An in vitro study . World Journal of Pharmacy and Pharmaceutical Sciences. 2016; 5(8): 289-300.
42. Mariana Yuriivna Kyznetsova, Olha Mycholaivna Makieieva, Dariia Oleksandrivna Livrovska, Maria Oleksandrivna Tymoshenko, Daryna Pavlivna Sheverova, Tetiana Ivanivna Halenova, Oleksiy Mycholayovych Savchuk, Lyudmila Ivanivna Ostapchenko. Effect of Aqueous Extract from *Phaseolus vulgaris* Pods on Lipid Peroxidation and Antioxidant Enzymes Activity in the Liver and Kidney of Diabetic Rats. Journal of Applied Pharmaceutical Science. 2015; 5 (05): 001-006.

43. Pradeepkumar MR, Joshi SD, Kulkarni VH, Savant C. Phytochemical screening and evaluation of analgesic and anti-inflammatory activities of *Phaseolus vulgaris linn*., seeds in rodents. Journal of Applied Pharmaceutical Science .2015; 5 (06): 066-069.
44. Sree Lakshmi N, Sujatha D, Bharathi K, Prasad KVSRG. Effect of *Phaseolus vulgaris* on in *vitro calcium* oxalate crystallization. Journal of Global Trends in Pharmaceutical Sciences. 2015; 6(3): 2817 – 2824.
45. Luka C D, Olatunde A, Tijjani H, Olisa-Enewe . Effect of aqueous extract of *Phaseolus vulgaris L.* (red kidney beans) on alloxan induced diabetic wistar rats. International Journal of Science Inventions Today. 2013; 2(4): 292-301.
46. Ocho-Anin Atchibri AL, Brou KD, Kouakou TH, Kouadio YJ and Gnakri D. Screening for antidiabetic activity and phytochemical constituents of common bean (*Phaseolus vulgaris L.*) seeds. Journal of Medicinal Plants Research. 2010; 4(17): 1757-1761.
47. Pari L, Venkateswaran S. Effect of an aqueous extract of *Phaseolus vulgaris* on plasma insulin and hepatic key enzymes of glucose metabolism in experimental diabetes. Pharmazie. 2003; 58: 916–919.
48. Hangen L and Bennink MR. Consumption of Black Beans and Navy Beans (*Phaseolus vulgaris)* Reduced Azoxymethane-Induced Colon Cancer in Rats. Nutrition and Cancer. 2002; 44(1): 60–65.
49. Venkateswaran S and Pari L. Antioxidant effect of *Phaseolus vulgaris* in streptozotocin-induced diabetic rats. Asia Pacific Journal. 2002; 11(3): 206–209.
50. Shashi Alok, Sanjay Kumar Jain, Amita Verma, Mayank Kumar, Monika Sabharwal. Pathophysiology of kidney, gallbladder and urinary stones treatment with herbal and allopathic medicine: A review. Asian Pacific Journal of Tropical Diseases. 2013; 3(6): 496-504.
51. Ikshit Sharma, Washim Khan, Rabea Parveen, Md. Javed Alam, Iftekhar Ahmad, Mohd Hafizur RehmanAnsari Sayeed Ahmad. Antiurolithiasis Activity of Bioactivity Guided Fraction of Bergenia ligulata against Ethylene Glycol Induced Renal Calculi in Rat. BioMed Research International . 2017; Article ID 1969525: 11 pages.
52. Salman Ahmed, Muhammad Mohtasheemul Hasan Zafar Alam Mahmood. Antiurolithiatic plants of family Fabaceae: A memoir of mechanism of action, therapeutic spectrum, formulations with doses . Journal of Pharmacognosy and Phytochemistry. 2017; 6(3): 592-596.
53. *Phaseolus vulgaris* -wikipedia. https://en.m.wikipedia.org/wiki/phaseolus-vulgaris.

54. Cranberry bean-Wikipedia . https://en.m.wikipedia.org/wiki/cranberry-bean.

55. Saleem ZM, Salman Ahmed and Muhammad Mohtasheemul Hasan. *Phaseolus vulgaris linn.* botany, medicinal uses, phytochemistry and pharmacology. World Journa lof Pharmaceutical Research 2016; 5(11): 1611-1616.
56. Rana Jenish H, Patel Urvi R, Sonia J. Patel, Vijay Lambole, Dhiren P. Shah. Pharmacological activities of *Phaseolus vulgaris*: a review. An International Journal of Pharmaceutical Sciences. 2016; 7(2): 107-115.
57. Nagesh Babu, Manchaladore N. Jyothi, Usha Shivaram, Sharadamma Narayanaswamy, Durg Vijay Rai, Varadahalli R. Devaraj. Identification mRNA from French Beans under Low NitrateStress. Turkish Journal of Biochemistry, 2014; 39(1):1-8.
58. Hangen L, Bennink MR. Consumption of black beans and navy beans (Phaseolus vulgaris) reduced azoxymethane-induced colon cancer in rats. Nutr Cancer 2002; 44: 60-5.
59. Savita Chaurasia, Rimsi Saxena. Biochemical Studies on Antioxidant Potential of Green Beans in Fresh and Processed Conditions. American Journal of Pharmatech Research. 2014; 4(3): 614-621.
60. Salma, Sadia Ghousia Baig, Muhammad Mohtasheemul Hasan, Salman Ahmed and Syeda Anum Fatima. Analgesic and Anti-Inflammatory Effects of *Phaseolus vulgaris L.* Fixed Oil in Rodents. Journal of Basic & Applied Sciences. 2018; 14: 174-179.
61. Dave Oomah B, Ame Lie Corbe, Parthiba Bala Subramanian. Antioxidant and Anti-inflammatory Activities of Bean *(Phaseolus vulgaris L.)* Hulls. Journal of Agricultural and Food Chemistry. 2010; 58: 8225–8230.
62. Janet Carrasco Castilla, Alan Javier Hernandez Alvarez, Cristian Jimenez Martinez, Carmen Jacinto Hernandez, Manuel Alaiz, Julio Giron Calle, Javier Vioque, Gloria Davila Ortiz. Anti-oxidant and metal chelating activities of *Phaseolus vulgaris L* . var. Jamapa protein isolates, phaseolin and lectin hydrolysate. Journal of Food Chemistry. 2012; 13: 1157-1164.
63. Radu Sumalan D, Camen, Carmen Dobrei. Salinity Tolerance in Some *Phaseolus vulgaris L.* Landraces from the Banat Area. 43rd Croatian and 3rd International Symposium on Agriculture. 2008; 371- 374.

64. Zongjian Zhu, Weiqin Jiang and Henry J. Thompson. Edible dry bean consumption (*Phaseolus vulgarisL.*) modulates cardiovascular risk factors and diet-induced obesity in rats and mice. British Journal of Nutrition. (2012); 108: S66–S73.
65. Ndeye Fatou Diaw Guene, Adama Diouf and Mamadou Gueye. Nodulation and nitrogen fixation of field grown common bean *(Phaseolus vulgaris*) as influenced by fungicide seed treatment. African Journal of Biotechnology. 2003: 2 (7): 198–201.
66. Amit Pawar , Nilofar Naikwade. Herbal Supplement of *Phaseolus vulgarisL.* Beans in Metabolic Abnormalities Induced by Atypical Antipsychotics: A Review. International Journal of Pharmaceutical & Biological Archives 2013; 4(4): 620 – 622.
67. Gary R, Takeoka, Lan Dao, Gerhard Full H, Rosalind Y, Wong Leslie A. Harden, Richard H. Edwards, Jose De J Berrios. Characterization of Black Bean (*Phaseolus vulgaris L.*) Anthocyanins. Journal of Agriculture and Food Chemistry. 1997; 45: 3395−3400.
68. Khandelwal K R. Text Book of Practical Pharmacognosy. 11 th ed. Pune: Nirali Prakashan. 2004; 149-155.
69. Trease G, Evans W. Textbook of Pharmacognosy, XIII Ed., Baillere Tindal, London. 1989; 799-803.
70. Kokate C.K. Practical Pharmacognosy, IV edition. Nirali Prakashan. 1994; 108-109. (1994).
71. Saumya S M, Mahaboob Basha P. *In vitro* Evaluation of Free Radical Scavenging Activities of *Panax ginseng* and *Lagerstremia specios* : A Comparative Analysis. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(1): 165-169.
72. Jayanthi P, Lalitha P. Reducing Power of the Solvent Extracts of *Eichhornia Crassipes* (Mart) Solms. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(3): 126-128.
73. Rohan Sharadan and Phatak, Asha Krishnaji Pratinidhi, Anup Subhash Hendre. Evaluation of antioxidant and free radical scavenging activities of spices mixture extract as additive with reference to synthetic antioxidant. Der Pharmacia Lettre. 2015; 7(2): 27-34.
74. Asante IK, Owusu E, Essilfie MK, Kwarteng M and Amuzuah O. Phytochemical investigation and thin layer chromatography of methanolic extracts of some selected grain legumes. Journal of Pharmacognosy and Phytochemistry. 2016; 5(3): 240-244.

75. Awah FM and Verla AW. Antioxidant activity, nitric oxide scavenging activity and phenolic contents of *Ocimum gratissimum* leaf extract. Journal of Medicinal Plants Research. 2010; 4(24): 2479-2487.
76. Khan SR. Reactive oxygen species, inflammation and calcium oxalate nephrolithiasis. Journal of Urology and Research. 2014; 3: 256-76.
77. Selvam R. Calcium oxalate stone disease: Role of lipid peroxidation and antioxidants. Journal of Urology and Research. 2002; 30: 35-47.