

Evaluation of Antinephrolithiatic activity of Ethanolic Extract of *Chenopodium album* Linn. (seed) in rat against ethylene glycol induced lithiasis.

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ABSTRACT –

Introduction:The Ayurveda has introduced a different Herbal plant can be used as a treatment of Nephrolithiasis. In our study we designed to evaluate prophylactic effect of 21 days treatment of ethanolic extract (50mg/kg&200mg/kg) of *Chenopodium album linn.* (seeds) in rats Against ethylene glycol (0.75% v/v)induced in lithiasis.

Materials and Methods:To induce nephrolithiasis, 0.75% v/v ethylene glycol was administered Intraperitoneal for 21 days. In our study we designed to evaluate prophylactic effect of 21 days treatment of ethanolic extract (50mg/kg &200mg/kg) of *Chenopodium album linn.* (seeds) in rats Against ethylene glycol (0.75%v/v)induced in lithiasis. To evaluate anti-nephrolithiasis, variation of the main risk factor (magnesium, calcium,urea, phosphate) has evaluate in 24 hours urine sample of rats. After completion of 21 days treatment body weight, food intakecalcium, and biochemical parameter Urea, Creatinine, albumin, globulin in blood serum. Lithiasis treatment with ethylene glycol caused damage of kidney, it metabolized the threetoxic like glycolate, glyoxalate which formed calcium oxalate crystal deposition in kidney and increased in hyperoxaluria, hypercalcinuria contributing to renal stone formation which was prevent by the regularly administration of EECA thereby reduce the growth of kidney Stone.

Result:The diseased Group II showed marked increase ($P < 0.001$ vs. normal Group I) in levels of urine calcium, uric acid,creatinine. Serum creatinine, BUN, Total protein,Albumin, Globulin levels were also increased. Histopathological studies of kidney sections revealed significant changes. Treatment with Ethanolic extract of *Chenopodium album* Lshowed significant ($P < 0.001$ vs. calculi-induced Group II) dose-dependent activity. A progressive increase in urine output, body weight, and decline in concentrations of

stone-forming components such as calcium, uric acid crystalwas observed.

Conclusion:It can be inferred that*Chenopodium album L* is effective in ethylene glycol-induced nephrolithiasis and may have a potential in preventing and curing nephrolithiasis.

KEYWORD- Antinephrolithiatic activity, Ethanolic Extract *Chenopodium album L*(EECA), Ethylene glycol, calcium oxalate crystal, Nephrolithiasis.

I. INTRODUCTION-

Approximately 20% of the population of humans suffers from nephrolithiasis, also known as urolithiasis, also known as kidney or renal stones. The production of stones is known as lithiasis, while solid nonmetallic minerals are known as urolithiasis [1]. Phosphates, oxalates, cystine, and uric acid make up the majority of the materials that go into urinary stones. These calculi contain calcium oxalate in around 80% of cases [2]. Stones form as a result of phase transition, in which dissolved salts turn solid due to oversaturation [3]. The most typical kidney stone type is calcium oxalate, which is one of several forms. These stones develop in the urinary tract through crystal nucleation, aggregation, and retention. Urinary stone obstruction may cause terrible agony.[4]. It has been shown that herbs have potent analgesic properties [5]. The treatment of individuals with stone illness frequently includes the prevention of recurrent stone development [6]. The most typical stones are those made of calcium, particularly calcium oxalates and phosphate [7]. According to Ayurveda, using plants medicinally reduces the likelihood of urolithiasis recurring while having no negative side effects [8]. Treatment options for calculi include surgery, lithotripsy, and local laser surgery to disrupt calculi [9]. Preventive treatment is necessary because these operations are pricey and recurrence is often [10]. The recurrence rate

can be decreased using phytotherapy [11]. In plants, antiurolithiatic action has been investigated. [12,13]. It takes time and an experimental investigation to validate natural medicines. The therapeutic potential of traditional medicinal herbs used to treat kidney stones has been made public in a number of pharmacological and clinical investigations using both in vitro and in vivo models [14].

*Chenopodium album*L, also called "bathua," "fathen," or "lamb's quarters," is a member of the Chenopodiaceae family. In many Asian nations, leaves are eaten like vegetables due to their great nutritional content. *C.album* has historically been used as a treatment for a variety of illnesses, including hepatic disorders[15, 16]. Chemical analysis of *C.album* leaves has revealed the presence of phenolics, sterols, vitamins, carotenoids, flavonoids, phytoecdysteroids, and minerals [17, 18]. Numerous *C. album* leaf extracts and compounds have been shown to have hypotensive [19], anti-inflammatory [20], anti-helminthic [21], and anticancer [22] properties. Recently, reports of *C.album*'s hepatoprotective efficacy against paracetamol-induced hepatotoxicity were made[23].

II. MATERIALS AND METHODS-

Plant Materials

Collection and Authentication of Plant Material-

The seed of *Chenopodium album* linn was collected from the local market of Ashoka Garden, Bhopal (M.P.). The authentication by Head of Department of Botany Dr. Saba Naaz, Professor of Saifia Science Collage Bhopal with Voucher Specimen No. 132/Saif/Sci/Bpl/2021.

Preparation of Plant extract-The *Chenopodium album* linn extracts are prepared in Hot extraction. The *Chenopodium album* linn seeds were collected and washed with clean water and dried at room temperature with a week and the seeds were powdered with the help of mixer and grinder. The powdered drug was sieved by sieve no. 24. The 100 gm of drug was packed inside the filter paper in the Soxhlet tube and introduced in the extraction unit of Soxhlet apparatus extractor and extraction was done with ethanol for 25 cycles. The extract was removed. The % yield of Ethanol extract was 7.65%. The concentrated crude drug extract was stored at 4°C in refrigerator and used for further study.

Phytochemical Screening-The existence of different primary and secondary metabolites was determined. The detection test for Alkaloids, glycosides, tannins, phenols, flavonoids, steroids etc. were carried out by the following standard methods.

Anti nephrolithiatic activity-

Considering various parameters, the Ethanol extract of *Chenopodium album* L was screened for its antinephrolithiatic potential.

Experimental animals study-30 Male and Female Albino Wistar Rat (150-200gm) were used in the present studies. All the experimental procedures and protocols used in this study were approved from the Institutional Animal Ethics Committee (TIP/IAEC/2021/06).

The animals were kept in polypropylene cages and maintained under standardized conditions (temp. 27°C ± 1°C, humidity 45-60% ± 4% and natural lighting) fed with a standard diet and water. The rats were divided into seven groups, a maximum of six animals were used in each group. They were housed seven per cage under standard laboratory conditions at room temperature at 22 ± 2°C with 12hr light/dark cycle. The animals were provided with pellet chow and water. Animals were acclimated to laboratory conditions one week prior to initiation of experiment.

Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Acute Oral Toxicity Studies-The acute oral toxicity study was carried out as per the guidelines set by the organization for economic co-operation and development (OECD) revised draft guidelines 423B received from the committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Acute oral dose toxicity studies were selected for the study were 100mg/kg, 200mg/kg, 300mg/kg for one day. Animals were observed for alterations in their behaviors. The animal was observed for 3 hours after administration and also 24 and 28 hours. All doses above *Chenopodium album* linn seed did not show any sign of toxicity up to the dose of 100 mg/kg. From acute toxicity test we conducted that (maximum dose 1/10th of maximum tolerable dose) i.e. (100 mg/kg) were found safe for the experiment.

Induction of Nephrolithiasis-Ethylene glycol (0.75% v/v) +Ammonium Chloride in drinking water was fed to all groups expect normal control for renal calculi till the 21th day.

Experimental procedure-The 30 healthy Female and male Wistar Albino rats weighing (150- 200 gm) were divide into 5 groups consisting of six animals in each group. Stones were induced in rats by giving 0.75% of ethylene glycol and 1% ammonium chloride in 100ml in drinking water and 1ml of drug is given by IP route. The inducing agent was administered for 21th days. In curative study two groups have involved in low dose 50 mg/kg and two groups have involved high dose 200mg/kg all groups except control, standard and negative control received extract by the IP route from 10th till 21th days.

Experimental Protocol:Animals were divided into 5 groups containing six animals in each group. During the experimental protocol, animals were allowed free access to food and water.

- Group I:Normal control animal
- Group II: Calculi-induced, received 0.75% ethylene glycol & Ammonium chloride in drinking water from 1st to 10th day.
- Group III:Standard drugcystone (750 mg/kg)10th to 21th day
- Group IV:EG+ Ethanolic extract(50mg/kg IP).
- Group V:EG+ Ethanolic extract(200mg/kg IP).

Biochemical parameter-

Urine Analysis:On 10th and 21th day, 24 h urine was collected of all the 5 groups, measured the urine volume, Calcium,uric acid, creatinine was recorded.

Serum Analysis: After the experimental period, the animals were sacrificed under anesthetic conditions and blood was collected. Serum was separated by centrifugation at 10,000 rpm for 10 min and analyzed for BUN, Creatinine, Total protein, Albumin,Globulin.

Statistical Analysis-Results were expressed in terms of mean \pm standard error mean. Differences among data were determined using one-way ANOVA test followed by Tukey multiple comparison test (GraphPad Software, Inc., Analystat, CA, USA.) and $P < 0.05$ was considered statistically significant.

Histopathological Studies- Examination of histopathological changes such as tubular congestion, tubular necrosis, glomerular congestion, peritubular inflammation, hemorrhage, and presence of calculi was done. The remaining kidney was embedded in liquid paraffin, 5 μ m sections were taken, stained with hematoxylin and eosin. The microscopic examination of thus prepared slides was done using compound microscope ($\times 50$). To determine the nephritic damage and recovery, photographs were taken.

III. RESULTS-

Phytochemical Screening- Phytochemical Screening EECA confirmed that presence of polyphenol, saponin, flavonoids, tannins, alkaloids, glycosides and steroids.

Acute Oral Toxicity-The acute oral toxicity (AOT) study of EECA was observed that it was safe up to 2000 mg/kg body weight and it was not showing any mortality, based on AOT selected dose for Antimutagenic activity are 50 mg/kg body weight and 200 mg/kg body weight.

Biological Parameter – Theantinephrolithiatic effects ofEthanolic extract of *Chenopodium album* L group were evaluated considering various parameters. In this study, ethylene glycol (0.75% v/v) in drinking water was administered to induce calculi except normal control Group I. The preliminary confirmation was done by doing urine microscopy where the presence of various stones was observed. As per the data reported, urine calcium, uric acid, and creatinine excretion significantly increased ($P < 0.001$) in calculi-induced (Group II) as compared to normal control (Group I). However, in both the preventive and curative dose regimen, there was an increased degree ($P < 0.001-0.05$) of reduction in the levels of urine calcium, oxalate, and phosphate, relevant to the dose of ethanolic extract administered, and highly significant decrease was observed in cystone treated group [Table 1, Group III to V]. Increase in dissolution and significant reduction ($P < 0.001$ vs. Group II) in the concentration of calcium, Creatinine BUN, total protein, albumin and globulin results in better prevention of stone formation.

Table No. 1 Effect of Ethanolic Extract of *Chenopodium album L.* against EG induced Nephrolithiasis. (Urine Volume)

S No.	Groups	1 st day	10 th day	21 st day
1.	Normal Control animal	2.86 ±0.004	2.87±0.89	2.88± 0.67
2.	Negativecontrol (EG+AC+ Drinking Water)	2.87± 0.04*a	2.55± 0.03*a	1.94± 0.076*a
3.	Standard Drug (EG+AC+Cystone750mg/kg)	2.86 ±0.087*b	2.67 ±0.05**b	2.79± 0.09**b
4.	EG+ EECA (50mg/kg IP)	2.87 ±0.078*a	2.54 ±0.04**b	2.58± 0.09*a
5.	EG+ EECA (200mg/kg IP)	2.89 ±0.08**b	2.57± 0.03*a	2.59 ±0.06**b

EECA= Ethanolic extract of *Chenopodium album L*
 AECA= Aqueous extract *Chenopodium album L*
 EG= Ethylene Glycol, IP= Intraperitoneal,
 AC=Ammonium Chloride
 All Value are expressed as Mean ±SEM

n=6 Wistar Albino Rat/group
 Comparison: a -Group I vs Group II
 b- Group II vs Group III, IV, V, VI&VII
 ***Significantly different with normal control,
 negative control and standard group at p<0.001.

Table No 2. Effect of Ethanolic Extract of *Chenopodium album L.* against EG induced Nephrolithiasis. (BUN)

S.No.	Groups	1 st day	10 th day	21 st day
1.	Normal Control animal	43.10±0.043	43.204±0.054	43.302±0.0067
2.	Negativecontrol (EG+AC+ Drinking Water)	43.08±0.09*a	49.69±0.004*a	49.50± 0.0078**a
3.	Standard Drug (EG+AC+Cystone750mg/kg)	43.09±0.007*b	45.504±0.05*b	44.905±0.0076*b
4.	EG+ EECA (50mg/kg IP)	43.12±0.003*b	47.64 ±0.03*b	48.68± 0.0054**a
5.	EG+EECA (200mg/kg IP)	43.15±0.005*a	46.23±0.76*a	46.334±0.0043*a

Table No 3. Effect of Ethanolic Extract of *Chenopodium album L.* against EG induced Nephrolithiasis.(Serum Creatinine)

S No	Group	1st	10th	21th
1.	Normal Control animal	1.43±0.112	1.43±0.267	1.43±0.345
2.	Negativecontrol (EG+AC +Drinking Water)	1.43±0.132**a	1.78±0.456*a	1.836 ±0.065**a
3.	Standard Drug (EG+ AC+Cystone750mg/kg)	1.43±0.321*b	1.73±0.332*b	1.489±0.007*b
4.	EG+ EECA (50mg/kg IP)	1.43±0.34*b	1.76±0.53**a	1.59±0.687*a

5.	EG+ EECA (200mg/kg IP)	1.44±0.001*a	1.74±0.76**a	1.58±0.332**b
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Table No 4. Effect of Ethanolic Extract of *Chenopodium album L.* against EG induced Nephrolithiasis. (Serum Total Protein)

S NO.	Groups	1 st day	10 th day	21 st day
1.	Normal Control animal	7.92±0.023	7.93±0.06	7.93±0.06
2.	Negativecontrol (EG+AC+ Drinking Water)	7.91±0.043*a	7.59±0.05*	7.48±0.054*a*b
3.	StandardDrug (EG+AC+Cystone750mg/kg)	7.93±0.065**b	7.88±0.05*a	7.90±0.05*b
4.	EG+ EECA (50mg/kg IP)	7.93±0.021*a	7.65±0.076**a	7.67±0.08*a
5.	EG+ EECA (200mg/kg IP)	7.91±0.054*a	7.69±0.054*b	7.69±0.07**a

Table No 5. Effect of Ethanolic Extract of *Chenopodium album L.* against EG induced Nephrolithiasis. (Serum Albumin)

S.No.	Groups	1 st day	10 th day	21 st day
1.	Normal Control animal	3.97±0.016	3.98±0.008	3.97±0.09
2.	Negativecontrol (EG+AC+Drinking Water)	3.97±0.06*a	3.75±0.076*a	3.62±0.007**a
3.	Standard Drug (EG+AC+ Cystone750mg/kg)	3.96±0.09**b	3.87±0.085*b	3.89±0.065*b
4.	EG+EECA(50mg/kg IP)	3.97±0.32**a	3.76±0.076**a	3.78±0.087**a
5.	EG+EECA (200mg/kg IP)	3.99±0.076*a	3.78±0.098*a	3.82±0.07**b

Table No 6. Effect of Ethanolic Extract of *Chenopodium album L.* against EG induced Nephrolithiasis. (Serum Globulin)

S.No.	Groups	1 st day	10 th day	21 st day
1.	Normal Control animal	3.95±0.018	3.96±0.08	3.96±0.09
2.	Negativecontrol (EG+AC+Drinking Water)	3.95±0.09*a	3.56±0.076**a	3.43±0.08*a
3.	StandardDrug (EG+AC+Cystone750mg/kg)	3.94±0.06**b	3.85±0.04*b	3.92±0.07*b
4.	EG+ EECA (50mg/kg IP)	3.95±0.43*b	3.82±0.09**b	3.86±0.06**a
5.	EG+ EECA (200mg/kg IP)	3.94±0.62*a	3.84±0.05*a	3.88±0.07*b

Table No7.Effect ofEthanolic Extract of *Chenopodium album L.* against EG induced Nephrolithiasis. (Urine Calcium)

S.No.	Groups	1 st day	10 th day	21 st day
1.	Normal Control animal	1.26±0.087	1.26±0.07	1.27±0.08
2.	Negativecontrol (EG+AC+ Drinking Water)	1.25±0.05**a	2.67±0.04*b	3.14±0.05**b
3.	Standard Drug (EG+AC+Cystone750mg/kg)	1.24±0.07*a	2.53±0.04*a	1.55±0.05*a
4.	EG+EECA (50mg/kg IP)	1.26±0.08**a	2.46±0.09*b	1.78±0.04*b
5.	EG+EECA (200mg/kg IP)	1.25±0.07*b	2.49±0.04**a	1.80±0.08*b

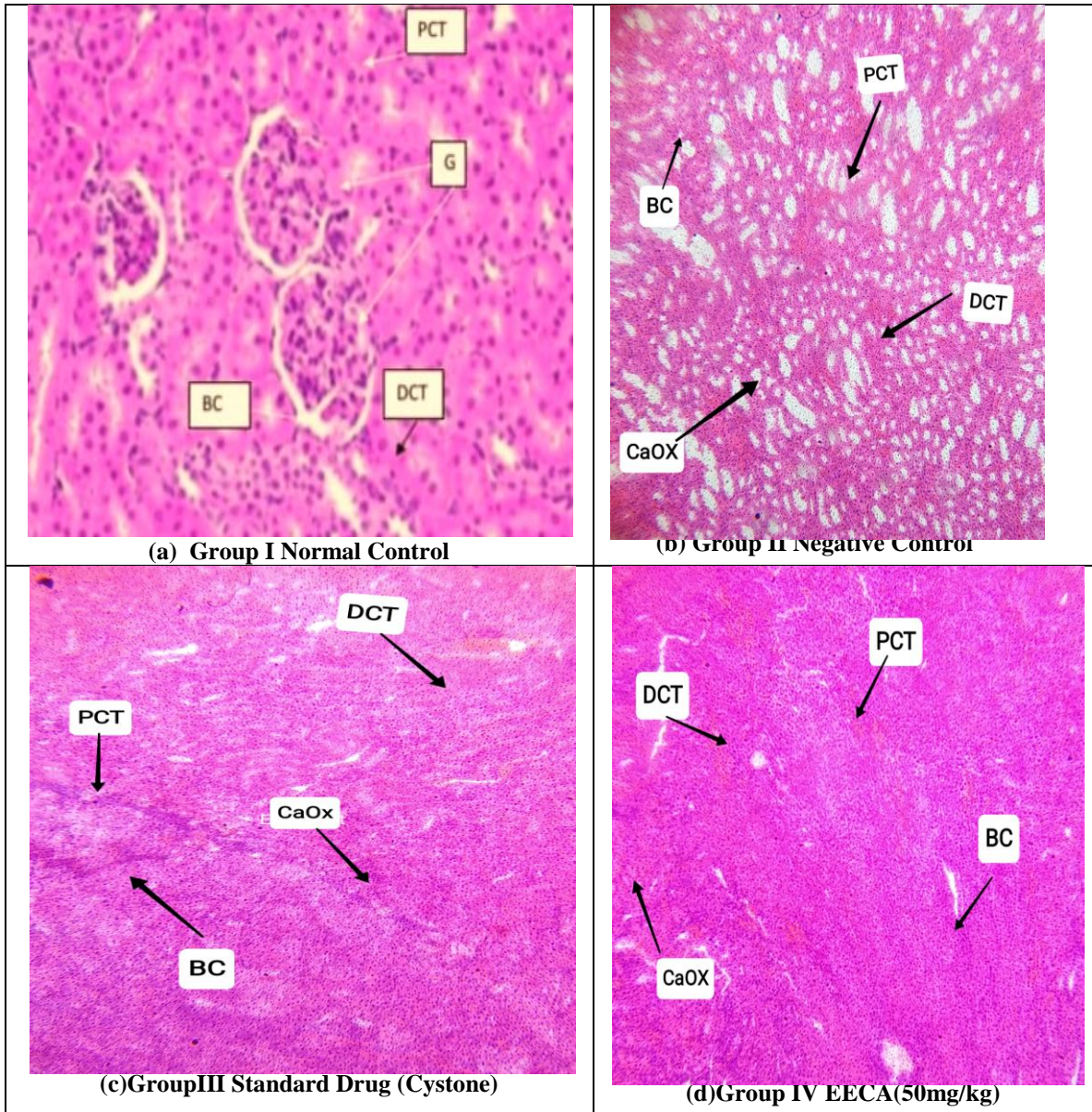
Table No 8.Effect ofEthanolic Extract of *Chenopodium album L.* against EG induced Nephrolithiasis. (Urine Creatinine)

S No	Groups	1 st day	10 th day	21 st day
1.	NormalControl animal	2.91±0.07	2.90±0.08	2.91±0.06
2.	Negativecontrol (EG+AC Drinking Water)	2.94±0.03**a	3.87±0.09*a	4.54±0.08*a
3.	Standard Drug (EG+AC+Cystone750mg/kg)	2.93±0.08*a	3.84±0.06**a	3.2±0.04*a
4.	EG+ EECA (50mg/kg IP)	2.93±0.03*b	3.75±0.06*a	3.34±0.02**b
5.	EG+ EECA (200mg/kg IP)	2.91±0.05*a	3.77±0.04*b	3.12±0.05*a

Table No.9Effect of Ethanolic Extract of *Chenopodium album L.* against EG induced Nephrolithiasis. (Uric Acid)

S No.	Groups	1 st day	10 th day	21 st day
1.	Normal Control animal	4.26±0.07	4.25±0.9	4.26±0.06
2.	Negativecontrol (EG+AC+ Drinking Water)	4.26±0.05*a	6.12±0.07**a	6.43±0.09*a
3.	Standard Drug (EG+AC+Cystone750mg/kg)	4.25±0.06*b	5.95±0.04*a	4.53±0.09**b
4.	EG+ EECA (50mg/kg IP)	4.26±0.06*a	5.87±0.04**b	5.34±0.05**b
5.	EG+ EECA (200mg/kg IP)	4.25±0.09**b	5.89±0.07*a	5.21±0.04*a

HISTOPATHOLOGY RESULTS



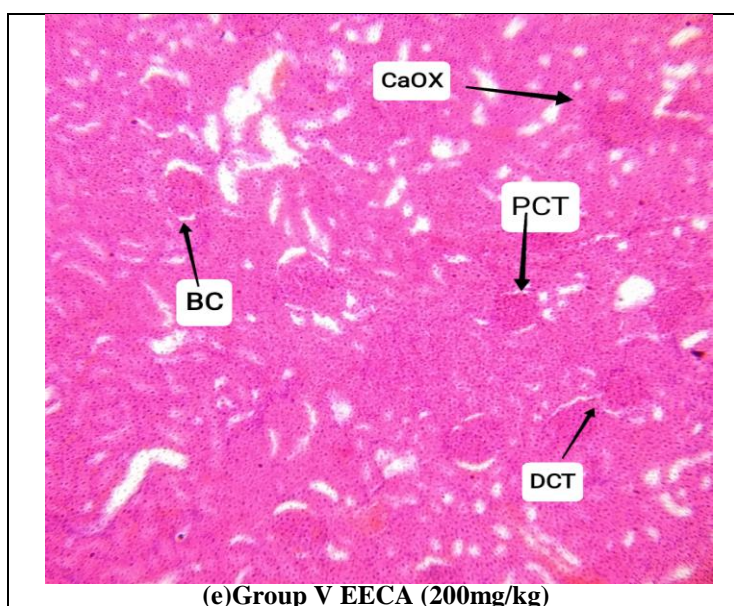


Figure 1 : Histological Examination of kidney section a) Group I Normal Control, (b) Group II Negative Control (c), Group III Standard Drug (Cystone), (d) Group IV EECA (50mg/kg), (e) Group V EECA (200mg/kg)

IV DISCUSSION-

Estimated life time risk of Urolithiasis in Asia 2%-5%, 8%-15% in Europe and America and around 20% in middle east. It is associated with a high risk of reoccurrence which is around 10%-23% per year 50% in 10 years and 75% in 20 years.

Stones may occur in any part of the urinary system like kidney, ureter, bladder, one of the most painful diseases. Urinary lithiasis is due to imbalance between inhibitors and promoters in the kidneys. Urinary super saturation is the driving force behind crystal formation in the kidneys. Since formation of crystalline particles must obviously start from super saturation. Super saturation is undoubtedly essential for stone formation.

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. Once a crystal nucleus has achieved a critical size and relative supersaturation remains above, overall free energy is decreased by adding new crystal components to the nucleus. This process is called crystal growth.

Aggregation is a process in which the salts in the solution stick together to form larger particles. Some researchers have 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;

crystal aggregation is the most important step in stone formation.

Chenopodium album linn. seeds are commonly used in the native system of medicine. Various parts of the plant like leaves and roots, flower are medicinally important. In order to investigate the medicinal use of *Chenopodium album linn. seeds* in nephrolithiasis, we evaluated crude extract for its antinephrolithiatic activity using different In vivo rat model of nephrolithiasis.

Calcification is a multifactorial phenomenon developing as a result of a cascade of events initiated by supersaturation, including crystal nucleation, growth, aggregation and retention Crystal inhibitors cystone like have been shown to decrease the saturation of CaOx and inhibit crystal nucleation, growth and aggregation, while reduced crystallization in urine of stone forming in rats. Interference with crystal growth and aggregation therefore seems a possible therapeutic strategy for the prevention of recurrent stone disease.

Preliminary Phytochemical analysis of ethanolic extract of *Chenopodium album linn.* had showed the presence of Phytoconstituents like alkaloids, flavonoids, tannins, saponins and Cardiac glycosides. Flavonoids and alkaloids are widely distributed in the plant which have the property to cure nephrolithiasis.

Due to this reason the plant has chosen to this study. This shows that the EECA may contain

substances that inhibit CaOx crystal aggregations and thus preventing a critical step in urinary stone formation, as larger particles are less likely to pass spontaneously in urinary tract. If the extract keeps CaOx particles dispersed in solution they can be easily eliminated. Decrease in body weight in ethylene glycol and ammonium chloride treated group was observed. There is no change in body weight in Cystone treated group and there was a significant Increase in body weight of EECA treated group given at both the doses of 50 mg/kg and 200 mg/kg.

On examining the renal function tests of ethylene glycol and ammonium chloride induced animals, the excretion of uric acid, creatinine and calcium has significantly increased with that of the control group. After treatment with the ethanolic & aqueous extract of *Chenopodium album* linn. (50 mg/kg and 200 mg/kg) the excretion of uric acid, urea, creatinine and calcium has significantly decreased.

Although the low dose was more potent than the high dose when compared with Cystone treated group, which is a standard. Ethanolic extract of *Chenopodium album* linn. has shown promising in vivo efficacy on nephrolithiasis, we have observed increase in the absorbance indicating the inhibition of Nucleation and Aggregation of calcium oxalate in in vivo studies.

For the in vivo antinephrolithic effect of EECA, 0.75% ethylene glycol (EG) and 1% ammonium chloride (AC)-induced hyperoxaluric rat model of nephrolithiasis was used. Since the stone inducing treatment, Ethylene glycol (EG), was given IP, therefore, the extract was given IP. in order to prevent any potential interaction of EG with plant constituents inside gut, interfering with absorption of either of the two.

Administration of EG and AC resulted in the increased CaOx crystalluria, with larger crystals due to hyperoxaluria, increase in water intake and urine output, which might be due to the renal impairment as evident by increase in serum creatinine, blood urea in lithiatic group as compared to normal group.

There was hypertrophy and extensive CaOx crystal deposition in kidneys of untreated rats. The renal tubules were markedly dilated, which might be due to the obstruction in distal renal tubular flow by large crystals Several in vivo and in vitro studies have demonstrated that hyperoxaluria, a major risk factor for calcium oxalate nephrolithiasis, results in greater production of superoxide and hydroxyl free radicals, leading to antioxidant imbalance, cell

membrane rupture and cell death.

which leads to CaOx crystal adherence and retention in renal tubules. Thus, it can be speculated that the inhibitory effect of the plant extract on CaOx crystal deposition in renal tubules is possibly caused by its antioxidant activity. The plant is considered relatively safer, as it has been used in different herbal preparations and supplements, is used in humans for ailment of diabetic and urinary disorders, which has also undergone clinical trials for several studies, with no reported side effect, for anti-inflammatory and nociceptive properties Thus, these data suggest that the effect of EECA in nephrolithiasis is mediated its effect through multiple pathways including inhibition of the CaOx crystal aggregation, which provide a step forward for designing further studies on EECA to establish its safety and efficacy for clinical use.

V. CONCLUSION-

The presented data indicate that administration of the ethanolic extracts of seed *Chenopodium album* Lto rats with ethylene glycol induced lithiasis reduced and prevented the growth of urinary stone, supportingfolk information regarding antinephrolithiatic activity of the plant. Result indicates alcoholic extract prevents renal stones. The mechanism underlying this effect may be due to increased diuresis and lowering the urinary concentrations of stone forming constituents. Further study is in progress for identification of the active constituents of the plant.

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