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ANTIUROLITHIATIC EFFECT OF HERBAL AND POLYHERBAL EXTRACTS AGAINST ETHYLENE GLYCOL INDUCED UROLITHIASIS IN WISTAR RAT

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ABSTRACT

This study is aimed to investigate the protective effect of Herbal and polyherbal formulation against ethylene glycol (EG) induced urolithiasis in Wistar rats. The protective effect was evaluated using EG-induced urolithiasis in rats. All the extracts were studied for in-vitro activity with the nucleation assay, Growth Assay, Cell Culture, Cytotoxicity - Trypan Blue Assay, LDH Leakage Assay for effective inhibition of crystal growth. The combination of extract shows better results for inhibition of growth. The results indicate that administration of extracts and inhibit the growth of urinary stones. It is also seen that the prophylactic effect is more efficient than the curative effect. Therefore, the extracts is useful to prevent the recurrence of urolithiasis as it proved its effect on the early stages of stone development. Related to increased diuresis and lowering of urinary concentrations of stone-forming components.

Keywords: Ethylene glycol, Wistar rats; Polyherbal formulation, Cytotoxicity -Trypan Blue Assay, LDH Leakage Assay, Urolithiasis.

1. Introduction

Urinary stone disease has affected humankind since antiquity and can persist, with serious medical consequences, throughout the patient's lifetime[1]. In addition, the incidence of kidney stones has been increased in western societies in the last five decades, in association with economic development. Most calculi in the urinary system arise from a common component of urine, calcium oxalate (CaO), representing up to 80% of analyzed stones. This may cause obstruction, hydronephrosis, infection, and hemorrhage in the urinary tract system[2-3]. Surgical operation, lithotripsy, and local calculus disruption using high-power laser are widely used to remove the calculi. Many remedies have been employed since ages to treat renal stones and most of them were from plants and proved to be useful[4].The present day medical management of nephrolithiasis is either costly or not without side effects[5]. The current study was aimed to evaluate the effectiveness of the Cissampelos pareira Linn, Biophytum sensitivum Linn, Fragaria vesva.Linn extracts on albino rats as a preventive agent against the development of kidney stones. he urolithiasis/nephrolithiasis suffering patients were treatedwith surgical

procedures which are invasive and expensive[6-7]. Thecalculi can be broken down with the help of percutaneousnephrolithotomy (PCNL) and extracorporeal shock Wave Litho-tripsy (ESWL) techniques. These tech-niques are less comfortable to patients and it will cause adverseeffects like haemorrhage, tubular necrosis and fibrosis to the kidney[8-9]. Those treatment proce-dures are expensive and the patients should have to follow upfor long period. So there is an urgent need to invent some moreclinically advanced anti-urolithiatic drugs that to halt the recur-rences, avert side effects and cost effective[10-13]. Theworld health organization (WHO) also showing interest towardsthe usage of herbal drugs/traditional medicines due to easy avail-ability, minimal cost and very low side effects. Cystone, a poly-herbal formulation was developed based on the reference foundin the ancient Ayurvedic system of medicine and widely utilizedfor an era to treat the urinary/renal calculi[14-15].

2. Materials and Methods

Acute toxicity study was carried out according to the OECD/OCDE, OECD Revised draft guidelines 423. Lethal dose of mice was calculated, 1/10th of this lethal dose was taken as an effective dose for subsequent studies. The effective doses (therapeutic doses), selected were:

Table 1.LD_{50} and the effective doses of C*issampelos pareira* L, *Fragaria vescaL, Biophytum sensitivum L.*

Sr.No	Extracts	LD_{50}	Effective dose
1	Ethanol extract of C*issampelos pareira* L, *Fragaria vescaL, Biophytum sensitivum L.* And combinations of extracts	4000mg/kg	400mg/kg B.W.
2	Isolate of C*issampelos pareira* L	4000mg/kg	400mg/kg B.W.
3	Isolate of *Fragaria vesca*	4000mg/kg	400mg/kg B.W.
4	Isolate of *Biophytum sensitivum L.*	4000mg/kg	400mg/kg B.W.

Waster albino rats about 150-200g were used in pharmacological studies. The animals were purchased from the national institute of biosciences, Dhangawadi. Institutional animal ethics committee (IAEC) approval (protocol no- RCP/IAEC/16-17/PO5) was obtained and care of the animals was taken as per guidelines of CPCSEA, ministry of social justice and empowerment, government of India. The animals had free access to standard diet with water supplied ad libitum under strict hygienic conditions. Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for 48 hr prior to experimental protocol to minimize if any of non specific stress. All the protocols and experiments were conducted in strict compliance.

Evaluation of Antiurolithiatic activity

Ethylene glycol and ammonium chloride induced hyperoxaluria model was used to induce urolithiasis. One hundred and two Wister Albino rats (180–250 g) were randomly divided into seventeen groups as Group I– XVII containing six animals in each. Group I served as a vehicle treated normal group and maintained on regular rat food and drinking water ad libitum and All remaining groups received calculi inducing treatment for 28 days, to induce urolithiasis for curative (CR) and preventive (PR) regimen. Groups IV, V, and VI served as CR, and groups VII, VIII, and IX as PR were treated with different extractsof C*issampelos pareira* L, *Fragaria vesca L, Biophytum sensitivum L.* Groups I, II, and III served as normal control, positive control (hyperurolithiatic), and standard (cystone 750 mg/kg) respectively. Oxalate, calcium, and phosphate were monitored in the urine and kidney. Serum, creatinine, and uric acid were also recorded. Group III received standard antiurolithiatic drug, cystone (750 mg/kg b.w.),Extract of C*issampelos pareira*L, *Fragaria vesca L, Biophytum sensitivum L.*, from 15th day till 28th day. Groups IV, VI, and VIII served as curative regimen (CR) 15th day till 28th day. Group V,VII, XI,XIII,XV, XVII received Extract of C*issampelos pareira* L, *Fragaria vescaL, Biophytum sensitivum L.*, from 1st day till 28th day and served as preventive regimen (PR). All extracts were given once daily.Comprised of 0.75% v/v ethylene glycol with 1% w/v ammonium chloride in drinking water for normal rats ad libitum for 3 days to accelerate lithiasis followed by 0.75% v/v ethylene glycol for 28 days. On 28 th day 24 hr after the treatment all the animals were hydrated with.

Protocol for activity

Group – I: Control

- Group – II: Ethylene glycol (0.75%) in drinking water + Vehicle
- Group – III: Ethylene glycol (0.75%) in drinking water + Cystone
- Group – IV: Ethylene glycol (0.75%) in drinking water + extract A – Curative study
- Group – V: Ethylene glycol (0.75%) in drinking water + extract A – Preventive study
- Group – VI: Ethylene glycol (0.75%) in drinking water + extract B – Curative study
- Group – VII: Ethylene glycol (0.75%) in drinking water + extract B– Preventive study
- Group – VIII: Ethylene glycol (0.75%) in drinking water + extract C – Curative study
- Group – IX: Ethylene glycol (0.75%) in drinking water + extract C – Preventive study
- Group – X: Ethylene glycol (0.75%) in drinking water + extract A+B – Curative study
- Group – XI: Ethylene glycol (0.75%) in drinking water + extract A +B– Preventive study
- Group – XII: Ethylene glycol (0.75%) in drinking water + extract A+C – Curative study
- Group – XIII: Ethylene glycol (0.75%) in drinking water + extract A +C- Preventive study
- Group – XIV: Ethylene glycol (0.75%) in drinking water + extract B +C- Curative study
- Group – XV: Ethylene glycol (0.75%) in drinking water + extract B +C- Preventive study
- Group – XVI: Ethylene glycol (0.75%) in drinking water + extract A+B +C- Curative study
- Group – XVII: Ethylene glycol (0.75%) in drinking water + extract A+B +C- Preventive study

(A- Extract of Cissampelos pareira linn,B- - Extract of Fragaria vesca linn C-- Extract of Biophytum sensitivum linn.)

Assessment of Antiurolithiatic Activity

Collection and analysis of urine

Rats were kept separately in metabolic cages and urine samples of 24 h were collected on 28th day. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. Urine samples were analyzed for calcium, phosphate, and oxalate content.

Figure 1. Effect of cystone and extracts

Effect of extracts and polyhedral combination on urine parameter in E.G 0.75%indused urolithiasis in rats.

Table 2. Mean standard ± deviation of Total Protein

Sr. No.	Sample Code	Total Protein (g/dl)
1	Control	10.29 ± 1.56$^{€}$
2	Normal	3.14 ± 1.17
3	CYS	5.10 ± 1.49$^{€}$
4	ECP CR	6.86 ± 0.92$^{€}$
5	EFV CR	7.01 ± 1.07$^{€}$
6	EBS CR	7.04 ± 1.48$^{€}$
7	ECP+EFV CR	5.74 ± 1.06$^{€}$
8	ECP+EBS CR	7.09 ± 1.46$^{€}$
9	EFV+EBS CR	5.67 ± 1.48$^{€}$
10	ECP+EFV+EBS CR	6.27 ± 1.70$^{€}$
11	ECP PR	7.13 ± 1.19$^{€}$
12	EFV PR	6.45 ± 0.95$^{€}$
13	EBS PR	6.32 ± 1.30$^{€}$
14	ECP+EFV PR	5.43 ± 0.83$^{€}$
15	ECP+EBS PR	6.50 ± 1.11$^{€}$
16	EFV+EBS PR	5.37 ± 0.62$^{€}$
17	ECP+EFV+EBS PR	5.71 ± 0.74$^{€}$

All Values are Mean □ S.E.M. (n=6); Significance values are (€) $P < 0.001$, (¥) $P < 0.01$ and (©) $P < 0.05$. Control group vs all groups by one way analysis variance test (ANOVA) followed by Dunnett's multiple comparison test.

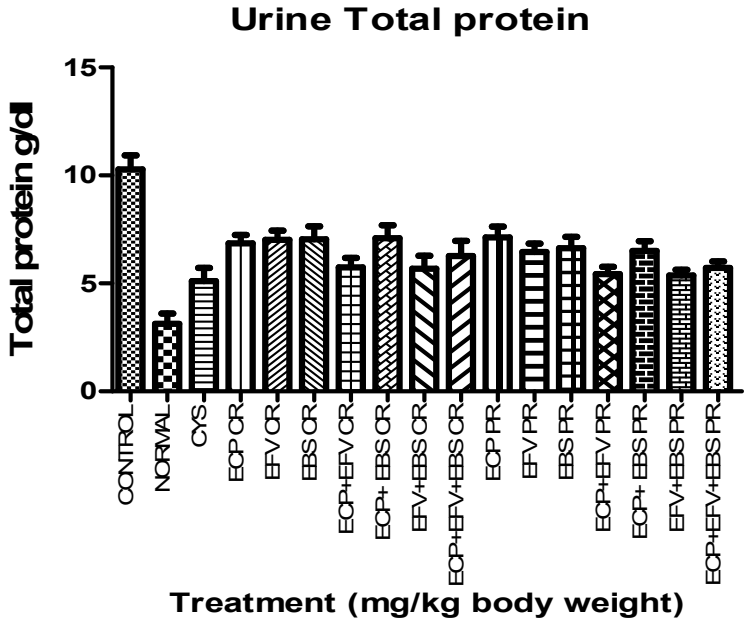

Figure 2. Graphical analysis of Total Protein

CYS- Cystone, ECP CR**-** Ethanol extract of C*issampelos pareira* L curative regimen**,** EFV CR , Ethanol extract of Fragaria vesca L curative regimen*,* EBS CR- Ethanol extract of *Biophytum sensitivum L* curative regimen *E*CP+EFV-CR - Ethanol extract of Combination of C*issampelos pareira* L and *Fragaria vesca L* curative regimen*,* ECP+EBS CR- C*issampelos pareira* and *Biophytum sensitivum* curative regimen*,*
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Table 3. Mean standard ± deviation of Urine Calcuim

Sr. No.	Sample Code	Urine Calcium (mg/dl)
1	Control	15.75 ± 1.91€
2	Normal	6.58 ± 1.04
3	CYS	6.02 ± 1.79€
4	ECP CR	9.36 ± 1.67€
5	EFV CR	7.52 ± 1.29€
6	EBS CR	7.31 ± 1.92€
7	ECP+EFV CR	7.11 ± 0.92€
8	ECP+EBS CR	6.69 ± 0.99€
9	EFV+EBS CR	7.03 ± 1.55€
10	ECP+EFV+EBS CR	7.52 ± 1.12€
11	ECP PR	7.45 ± 1.09€
12	EFV PR	6.82 ± 1.48€
13	EBS PR	6.44 ± 2.26€
14	ECP+EFV PR	6.88 ± 0.62€
15	ECP+EBS PR	6.40 ± 0.75€
16	EFV+EBS PR	6.67 ± 0.69€
17	ECP+EFV+EBS PR	5.62 ± 0.46€

All Values are Mean □ S.E.M. (n=6); Significance values are (€) $P < 0.001$, (¥) $P < 0.01$ and (©) $P < 0.05$. Control group vs all groups by one way analysis variance test (ANOVA) followed by Dunnett's multiple comparison test.

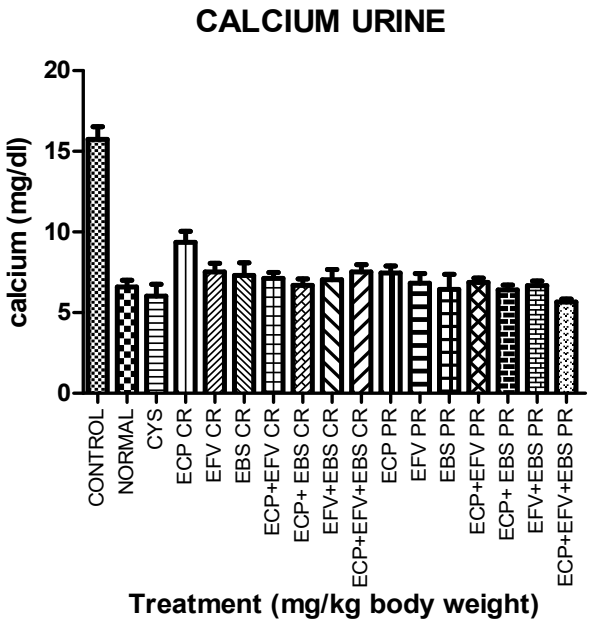

Figure 3. Graphical analysis of Urine Calcium

CYS- Cystone, ECP CR- Ethanol extract of C*issampelos pareira* L curative regimen**,** EFV CR , Ethanol extract of Fragaria vesca L curative regimen*,* EBS CR- Ethanol extract of *Biophytum sensitivum L* curative regimen *E*CP+EFV-CR - Ethanol extract of Combination of C*issampelos pareira* L and *Fragaria vesca L* curative regimen*,* ECP+EBS CR- C*issampelos pareira* and *Biophytum sensitivum* curative regimen*,*
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Table 4. Mean standard ± deviation of Urine Creatinine

Sr. No.	**Sample Code**	**Urine Creatinine (mg/dl)**
1	Control	10.31 ± 1.06€
2	Normal	3.98 ± 0.94
3	CYS	5.32 ± 0.77€
4	ECP CR	7.56 ± 0.37€
5	EFV CR	7.21 ± 0.47€
6	EBS CR	6.95 ± 0.55€
7	ECP+EFV CR	6.47 ± 0.36€
8	ECP+EBS CR	6.83 ± 0.79€
9	EFV+EBS CR	6.83 ± 1.18€
10	ECP+EFV+EBS CR	6.25 ± 0.99€
11	ECP PR	6.64 ± 1.22€
12	EFV PR	6.29 ± 0.98€
13	EBS PR	5.93 ± 0.81€
14	ECP+EFV PR	7.44 ± 1.36€
15	ECP+EBS PR	6.67 ± 1.41€
16	EFV+EBS PR	5.65 ± 1.03€
17	ECP+EFV+EBS PR	4.81 ± 0.86€

*All Values are Mean □ S.E.M. (n=6); Significance values are (€) $P < 0.001$, (¥) $P < 0.01$ and (©) $P < 0.05$. Control group vs all groups by one way analysis variance test (ANOVA) followed by

Dunnett's multiple comparison test.ns = non significant

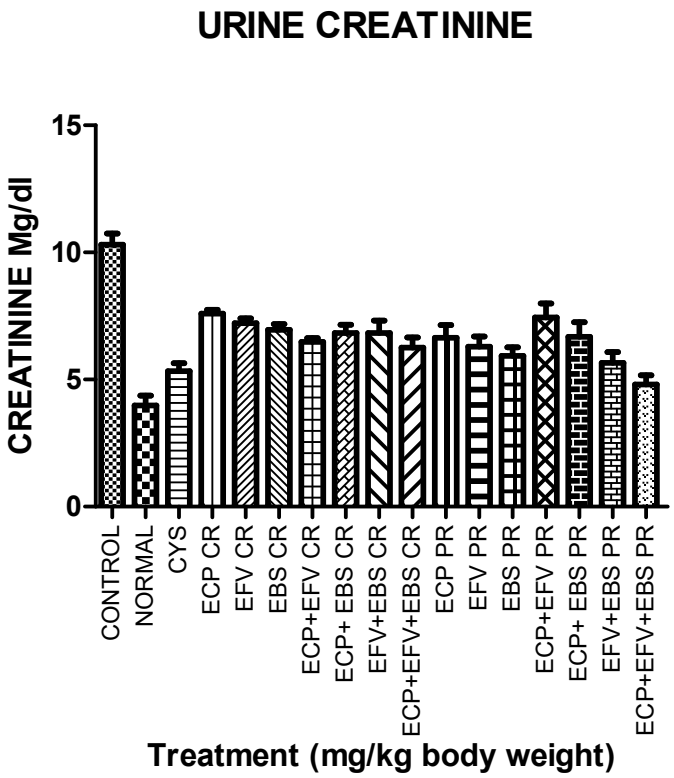

Figure 4. Graphical analysis of Urine Creatinine

CYS- Cystone, ECP CR**-** Ethanol extract of C*issampelos pareira* L curative regimen**,** EFV CR , Ethanol extract of Fragaria vesca L curative regimen*,* EBS CR- Ethanol extract of *Biophytum sensitivum L* curative regimen *E*CP+EFV-CR - Ethanol extract of Combination of C*issampelos pareira* L and *Fragaria vesca L* curative regimen*,* ECP+EBS CR- C*issampelos pareira* and *Biophytum sensitivum* curative regimen*,*

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EFV+EBS Pr - *Fragaria vesca* Land *Biophytum sensitivum* Lprotective regimen*,* ECP+EFV+EBS PR- C*issampelos pareira* L, *Fragaria vesca* L *andBiophytum sensitivum* Lprotective regimen.

Table 5. Mean standard ± deviation of Urine Oxalate

Sr. No.	Sample Code	Urine Oxalate (mg/dl)
1	Control	20.03 ± 2.84€
2	Normal	4.41 ± 1.46
3	CYS	5.37 ± 1.45€
4	ECP CR	12.67 ± 1.58€
5	EFV CR	11.67 ± 1.39€
6	EBS CR	11.25 ± 1.59€
7	ECP+EFV CR	10.82 ± 0.97€
8	ECP+EBS CR	10.54 ± 2.09€
9	EFV+EBS CR	11.04 ± 2.11€
10	ECP+EFV+EBS CR	9.37 ± 1.25€
11	ECP PR	10.62 ± 2.41€
12	EFV PR	11.80 ± 3.01€
13	EBS PR	10.03 ± 2.29€
14	ECP+EFV PR	10.49 ± 0.78€
15	ECP+EBS PR	9.73 ± 1.31€
16	EFV+EBS PR	9.91 ± 1.34€
17	ECP+EFV+EBS PR	8.13 ± 1.41€

All Values are Mean □ S.E.M. (n=6); Significance values are (€) $P < 0.001$, (¥) $P < 0.01$ and (©) $P < 0.05$. Control group vs all groups by one way analysis variance test (ANOVA) followed by Dunnett's multiple comparison test.

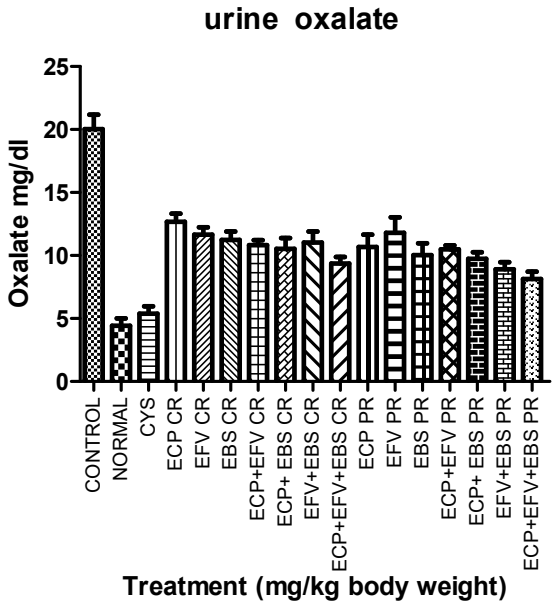

Figure 5. Graphical analysis of Urine Oxalate

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Table 6. Mean standard ± deviation of Urine Phosphates

Sr. No.	**Sample Code**	**Urine Phosphates (mg/dl)**
1	Control	8.04 ± 0.77$^{€}$
2	Normal	3.45 ± 0.51
3	CYS	4.10 ± 0.46$^{€}$
4	ECP CR	7.42 ± 0.59 ^{ns}
5	EFV CR	7.90 ± 0.60 ^{ns}
6	EBS CR	7.36 ± 0.97 ^{ns}
7	ECP+EFV CR	6.15 ± 1.07 ns
8	ECP+EBS CR	5.55 ± 1.59 ^{ns}
9	EFV+EBS CR	6.54 ± 1.61$^{¥}$
10	ECP+EFV+EBS CR	6.37 ± 1.87 ^{ns}
11	ECP PR	6.97± 0.49 ^{ns}
12	EFV PR	6.78± 0.34 ^{ns}
13	EBS PR	6.69± 0.65 ^{ns}
14	ECP+EFV PR	7.45 ± 0.43 ^{ns}
15	ECP+EBS PR	7.37 ± 0.99 ^{ns}
16	EFV+EBS PR	6.96 ± 1.42 ^{ns}
17	ECP+EFV+EBS PR	5.16 ± 1.56$^{€}$

* All Values are Mean □ S.E.M. (n=6); Significance values are $^{(€)}$ *P* < 0.001, $^{(¥)}$ P < 0.01 and $^{(©)}$ P < 0.05. Control group vs all groups by one way analysis variance test (ANOVA) followed by Dunnett's multiple comparison test.

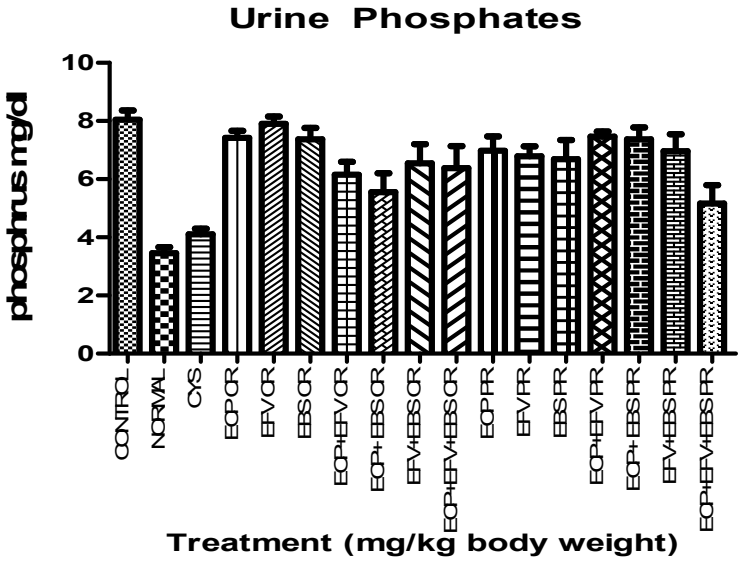

Figure 6. Graphical analysis of urine phosphates

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Table 7. Mean ± standard deviation of Urine Urea

Sr. No.	**Sample Code**	**Urea (mg/dl)**
1	Control	587.80 ± 92.06$^{€}$
2	Normal	99.88 ± 34.03
3	CYS	136.30 ± 37.24$^{€}$
4	ECP CR	382.30 ± 46.15$^{€}$
5	EFV CR	160.80 ± 60.65$^{€}$
6	EBS CR	256.40 ± 81.94$^{€}$
7	ECP+EFV CR	212.80 ± 54.47$^{€}$

8	ECP+EBS CR	246.00 ± 71.48$^{€}$
9	EFV+EBS CR	179.30 ± 21.14$^{€}$
10	ECP+EFV+EBS CR	157.70 ± 13.30$^{€}$
11	ECP PR	345.50 ± 64.38$^{€}$
12	EFV PR	177.60 ± 50.76$^{€}$
13	EBS PR	219.20 ± 63.20$^{€}$
14	ECP+EFV PR	241.10 ± 23.39^{ns}
15	ECP+EBS PR	193.40 ± 10.92 ^{ns}
16	EFV+EBS PR	160.20 ± 23.87 ^{ns}
17	ECP+EFV+EBS PR	133.00 ± 12.74 ^{ns}

All Values are Mean □ S.E.M. (n=6); Significance values are $^{(€)}$ $P < 0.001$, $^{(¥)}$ $P < 0.01$ and $^{(©)}$ $P < 0.05$. Control group vs all groups by one way analysis variance test (ANOVA) followed by Dunnett's multiple comparison test.

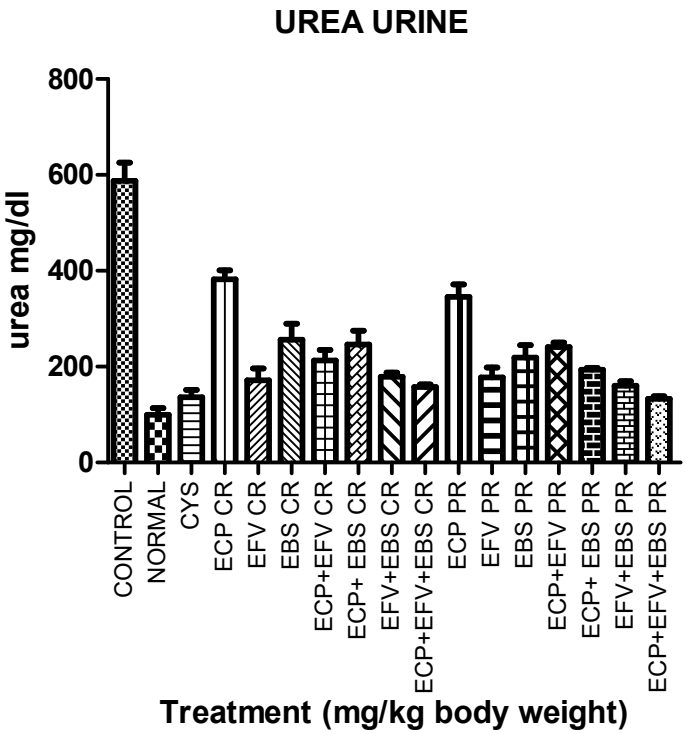


Figure 7. Graphical analysis of Urine Urea

Table 8. Mean ± standard deviation of Urine Uric acid

Sr. No.	**Sample Code**	**Uric acid (mg/dl)**
1	Control	10.73 ± 1.12$^{€}$
2	Normal	5.83 ± 0.52
3	CYS	7.54 ± 1.02$^{€}$

4	ECP CR	7.78 ± 1.57€
5	EFV CR	7.57 ± 1.88€
6	EBS CR	7.43 ± 1.12€
7	ECP+EFV CR	8.23 ± 0.82¥
8	ECP+EBS CR	8.04 ± 0.51€
9	EFV+EBS CR	7.13 ± 0.73€
10	ECP+EFV+EBS CR	6.75 ± 0.46€
11	ECP PR	8.19 ± 1.39¥
12	EFV PR	7.90 ± 1.88€
13	EBS PR	7.71 ± 1.13€
14	ECP+EFV PR	8.35 ± 0.49¥
15	ECP+EBS PR	7.60 ± 0.75€
16	EFV+EBS PR	6.92 ± 0.87€
17	ECP+EFV+EBS PR	6.52 ± 0.51€

* All Values are Mean □ S.E.M. (n=6); Significance values are (€) $P < 0.001$, (¥) $P < 0.01$ and (©) $P < 0.05$. Control group vs all groups by one way analysis variance test (ANOVA) followed by Dunnett's multiple comparison test.

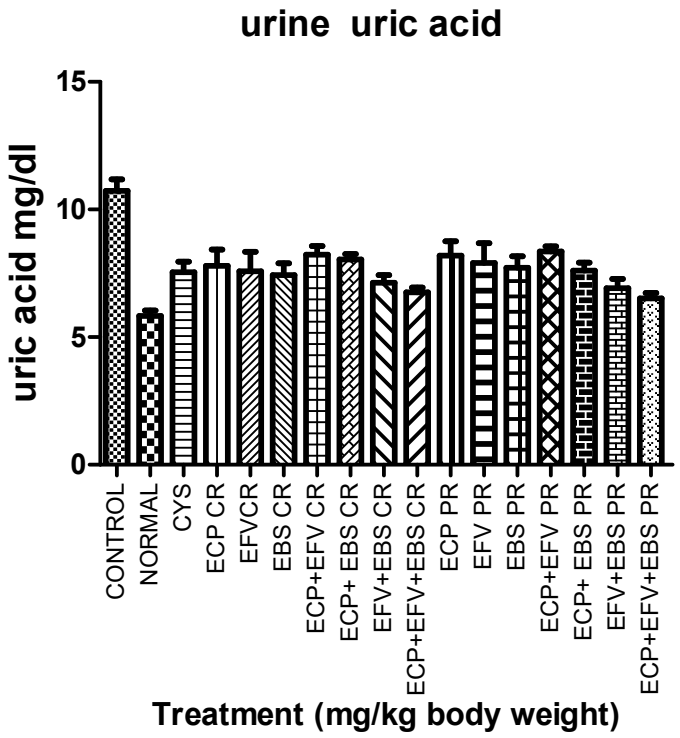


Figure 8. Graphical analysis of Urine Uric Acid

CYS- Cystone, ECP CR- Ethanol extract of C*issampelos pareira* L curative regimen**,** EFV CR , Ethanol extract of Fragaria vesca L curative regimen*,* EBS CR- Ethanol extract of *Biophytum sensitivum L* curative regimen *E*CP+EFV-CR - Ethanol extract of Combination of

C*issampelos pareira* L and *Fragaria vesca L* curative regimen, ECP+EBS CR- C*issampelos pareira* and *Biophytum sensitivum* curative regimen,
EFV+EBS CP- *Fragaria vesca* Land *Biophytum sensitivum* Lcurative regimen, ECP+EFV+EBS CR- C*issampelos pareira* L, *Fragaria vesca* L *andBiophytum sensitivum* Lcurative regimen.

ECP PR- Ethanol extract of C*issampelos pareira* L protective regimen, EFV PR , Ethanol extract of Fragaria vesca L protective regimen, EBS PR- Ethanol extract of *Biophytum sensitivum L* protective regimen *E*CP+EFV-PR - Ethanol extract of Combination of C*issampelos pareira* L and *Fragaria vesca L* Protective regimen, ECP+EBS PR- C*issampelos pareira* and *Biophytum sensitivum* protective regimen,
EFV+EBS PR - *Fragaria vesca* Land *Biophytum sensitivum* Lprotective regimen, ECP+EFV+EBS PR- C*issampelos pareira* L, *Fragaria vesca* L *andBiophytum sensitivum* Lprotective regimen.

At the end of urinary biochemical data that were obtained at the end of the experiments in each group. In the present study chronic administration of 0.75%(V/V) ethylene glycol aqueous solution to male waster rats resulted in hyperoxaluria, there was an in urinary calcium, uric acid , urea, and oxalate in calculi induced animals as in group I, however , supplementation with ECP CR- Ethanol extract of C*issampelos pareira* L curative Regimen, EFV CR , Ethanol extract of Fragaria vesca L curative Regimen, BS CR- Ethanol extract of *Biophytum sensitivum L curative* Regimen *E*CP+EFV-CR - Ethanol extract of Combination of C*issampelos pareira* L and - *Fragaria vesca L curative* Regimen, *E*CP+EBS CR- C*issampelos pareira* and *Biophytum sensitivum* Curative regimen, *E* FV+EBS CP- *Fragaria vesca L* and *Biophytum sensitivum* Curative Regimen ,ECP+EFV+EBS CR- C*issampelos pareira*, *Fragaria vesca* and *Biophytum sensitivum* Curative Regimen. As well as in ECP PR- Ethanol extract of C*issampelos pareira* Lprotective Regimen, EFV PR , Ethanol extract of Fragaria vescaprotective Regimen, BS PR- Ethanol extract of *Biophytum sensitivum L* protectiveRegimen *E*CP+EFV-PR - Ethanol extract of Combination of C*issampelos pareira* L and - *Fragaria vesca* protective Regimen, *E*CP+EBS PR- C*issampelos pareira* and *Biophytum sensitivum* protective Regimen , *E* FV+EBS PR - *Fragaria vescaL* and *Biophytum sensitivum* protective Regimen ,ECP+EFV+EBS PR- C*issampelos pareira*, *Fragaria vesca L andBiophytum sensitivum* protectiveRegimen. Significantly ($P<0.05$) inhibited these changes in urinary calcium, uric acid, urea and oxalate excretion dose dependant in both curative and preventive regimen. In all the best results were showed by combination therapy of, ,ECP+EFV+EBS CR- C*issampelos pareira*, *Fragaria vesca L andBiophytum sensitivum* Curative Regimen.ECP+EFV+EBS PR- C*issampelos pareira*, *Fragaria vesca L andBiophytum sensitivum* protectiveRegimen.As compaired with standard cystone drug.

Serum Analysis- At the end of the experiments, blood samples were collcted from the retro-orbital plexus under anesthetic conditions and analyzed for creatinine, urea and uric acid

Table 9. Mean standard ± deviation of Serum creatinine

Sr.	Sample Code	Serum creatinine

No.		(mg/dl)
1	Control	1.06± 0.054€
2	Normal	0.6262±0.02
3	CYS	0.6792±0.031€
4	ECP CR	0.8552±0.040¥
5	EFV CR	0.8308±0.040€
6	EBS CR	0.7580 ± 0.031€
7	ECP+EFV CR	0.8167 ± 0.026€
8	ECP+EBS CR	0.8165 ± 0.029€
9	EFV+EBS CR	0.8405 ± 0.053¥
10	ECP+EFV+EBS CR	0.7518 ± 0.030€
11	ECP PR	0.8468 ± 0.054¥
12	EFV PR	0.7542 ± 0.029€
13	EBS PR	0.6602 ± 0.052€
14	ECP+EFV PR	0.7688 ± 0.030€
15	ECP+EBS PR	0.7247 ± 0.026€
16	EFV+EBS PR	0.8068 ± 0.052€
17	ECP+EFV+EBS PR	0.6313 ± 0.018€

All Values are Mean □ S.E.M. (n=6); Significance values are (€) $P < 0.001$, (¥) $P < 0.01$ and (©) $P < 0.05$. Control group vs all groups by one way analysis variance test (ANOVA) followed by Dunnett's multiple comparison test.

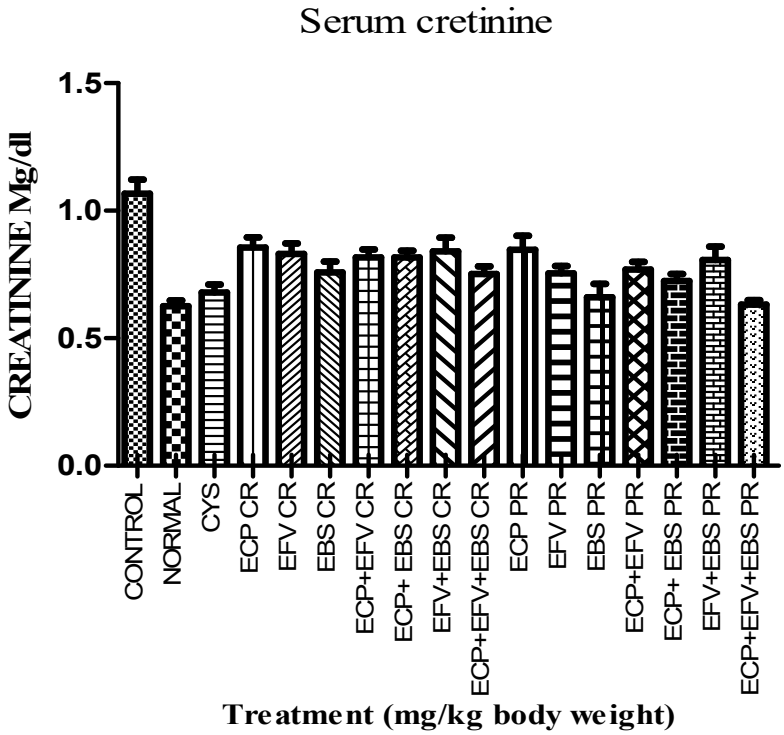


Figure 9. Graphical analysis of mean standard ± deviation of Serum creatinine

CYS- Cystone, ECP CR- Ethanol extract of C*issampelos pareira* L curative regimen**,** EFV CR , Ethanol extract of Fragaria vesca L curative regimen*,* EBS CR- Ethanol extract of *Biophytum sensitivum L* curative regimen *E*CP+EFV-CR - Ethanol extract of Combination of C*issampelos pareira* L and *Fragaria vesca L* curative regimen*,* ECP+EBS CR- C*issampelos pareira* and *Biophytum sensitivum* curative regimen*,*
EFV+EBS CP- *Fragaria vesca* Land *Biophytum sensitivum* Lcurative regimen*,* ECP+EFV+EBS CR- C*issampelos pareira* L, *Fragaria vesca* L *andBiophytum sensitivum* Lcurative regimen.
ECP PR- Ethanol extract of C*issampelos pareira* L protective regimen**,** EFV PR , Ethanol extract of Fragaria vesca L protective regimen*,* EBS PR- Ethanol extract of *Biophytum sensitivum L* protective regimen *E*CP+EFV-PR - Ethanol extract of Combination of C*issampelos pareira* L and *Fragaria vesca L* Protective regimen*,* ECP+EBS PR- C*issampelos pareira* and *Biophytum sensitivum* protective regimen*,*
EFV+EBS PR - *Fragaria vesca* Land *Biophytum sensitivum* Lprotective regimen*,* ECP+EFV+EBS PR- C*issampelos pareira* L, *Fragaria vesca* L *andBiophytum sensitivum* Lprotective regimen.

Table 10. Mean standard ± deviation of Serum phosphorus

Sr. No.	**Sample Code**	**Serum phosphorus (mg/dl)**
1	Control	2.048±0.36$^{€}$
2	Normal	0.6173±0.26
3	CYS	0.7715±0.14$^{€}$
4	ECP CR	1.253±0.30$^{€}$
5	EFV CR	0.9158±0.087$^{€}$
6	EBS CR	1.036 ±0.40$^{€}$
7	ECP+EFV CR	1.306± 0.40$^{€}$
8	ECP+EBS CR	0.7618±0.16$^{€}$
9	EFV+EBS CR	0.8055±0.087$^{€}$
10	ECP+EFV+EBS CR	0.8205±0.11$^{€}$
11	ECP PR	1.151±0.36$^{€}$
12	EFV PR	0.7620± 0.15$^{€}$
13	EBS PR	0.7358±0.19$^{€}$
14	ECP+EFV PR	0.7947±0.15$^{€}$
15	ECP+EBS PR	0.8292±0.11$^{€}$
16	EFV+EBS PR	0.7055±0.094$^{€}$
17	ECP+EFV+EBS PR	0.5952±0.061$^{€}$

All Values are Mean □ S.E.M. (n=6); Significance values are $^{(€)}$ $P < 0.001$, $^{(¥)}$ $P < 0.01$ and $^{(©)}$ $P < 0.05$. Control group vs all groups by one way analysis variance test (ANOVA) followed by

Dunnett's multiple comparison test.

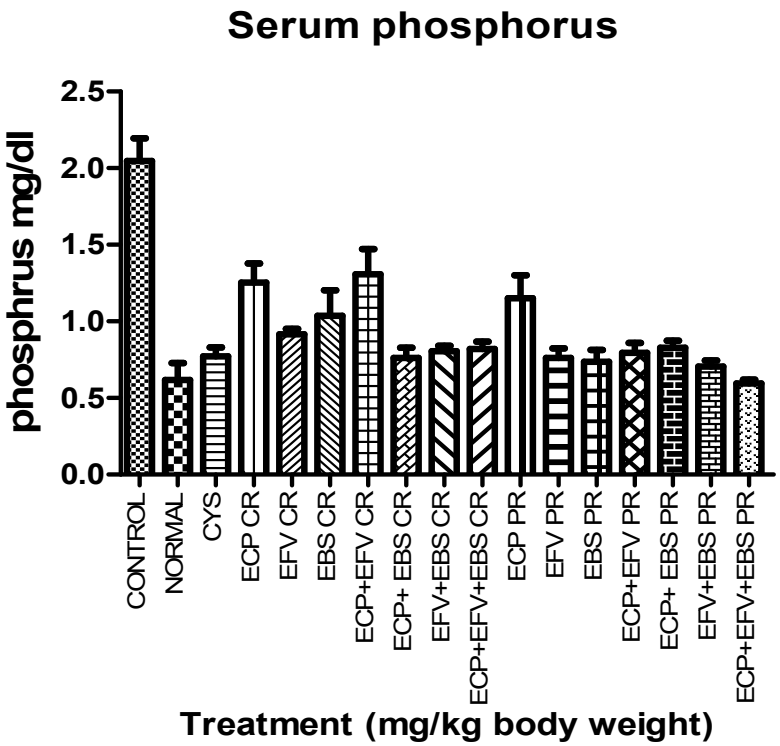


Figure 10. Graphicalanalysis of mean standard ± deviation of Serum phosphorus

CYS- Cystone, ECP CR**-** Ethanol extract of C*issampelos pareira* L curative regimen**,** EFV CR , Ethanol extract of Fragaria vesca L curative regimen*,* EBS CR- Ethanol extract of *Biophytum sensitivum L* curative regimen *E*CP+EFV-CR - Ethanol extract of Combination of C*issampelos pareira* L and *Fragaria vesca L* curative regimen*,* ECP+EBS CR- C*issampelos pareira* and *Biophytum sensitivum* curative regimen*,*
EFV+EBS CP- *Fragaria vesca* Land *Biophytum sensitivum* Lcurative regimen*,* ECP+EFV+EBS CR- C*issampelos pareira* L, *Fragaria vesca* L *andBiophytum sensitivum* Lcurative regimen.
ECP PR**-** Ethanol extract of C*issampelos pareira* L protective regimen**,** EFV PR , Ethanol extract of Fragaria vesca L protective regimen*,* EBS PR- Ethanol extract of *Biophytum sensitivum L* protective regimen *E*CP+EFV-PR - Ethanol extract of Combination of C*issampelos pareira* L and *Fragaria vesca L* Protective regimen*,* ECP+EBS PR- C*issampelos pareira* and *Biophytum sensitivum* protective regimen*,*
EFV+EBS PR - *Fragaria vesca* Land *Biophytum sensitivum* Lprotective regimen*,* ECP+EFV+EBS PR- C*issampelos pareira* L, *Fragaria vesca* L *andBiophytum sensitivum* Lprotective regimen.

Table 11. Mean standard ± deviation of Serum urea (mg/dl)

Sr. No.	Sample Code	Serum urea (mg/dl)
1	Control	58.33± 3.409€
2	Normal	32.05±5.125
3	CYS	34.74±1.990€
4	ECP CR	48.26±1.578©
5	EFV CR	46.15±2.433€
6	EBS CR	36.54±2.455€
7	ECP+EFV CR	44.74± 3.355€
8	ECP+EBS CR	40.53 ±2.660€
9	EFV+EBS CR	35.64± 2.341€
10	ECP+EFV+EBS CR	35.41±1.155€
11	ECP PR	44.74±3.355€
12	EFV PR	40.53±2.660€
13	EBS PR	35.64±2.341€
14	ECP+EFV PR	42.64±1.154€
15	ECP+EBS PR	37.09± 1.380€
16	EFV+EBS PR	29.48± 1.046€
17	ECP+EFV+EBS PR	27.04±1.911€

All Values are Mean □ S.E.M. (n=6); Significance values are (€) $P < 0.001$, (¥) $P < 0.01$ and (©) $P < 0.05$. Control group vs all groups by one way analysis variance test (ANOVA) followed by Dunnett's multiple comparison test.

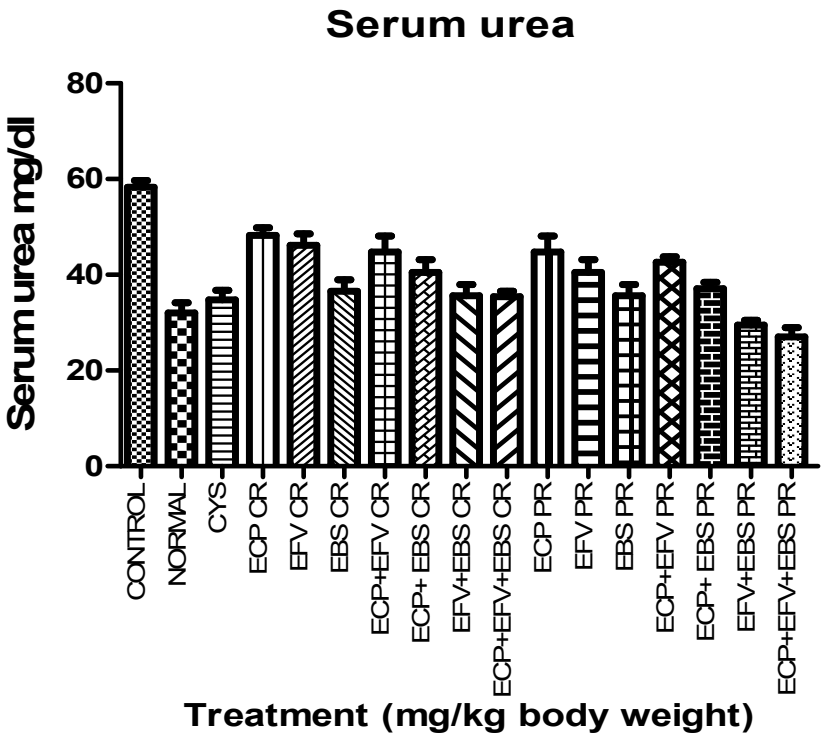


Figure 10. Graphical analysis of Mean standard ± deviation of Serum urea

Table 11. Mean standard ± deviation of Serum uric acid (mg/dl)

Sr. No.	Sample Code	Serum uric acid (mg/dl)
1	Control	5.205± 0.56€
2	Normal	1.852±0.24
3	CYS	3.278± 0.20€
4	ECP CR	3.554± 0.41¥
5	EFV CR	3.335± 0.32¥
6	EBS CR	3.130± 0.34€
7	ECP+EFV CR	3.077± 0.29€
8	ECP+EBS CR	2.899± 0.19€
9	EFV+EBS CR	2.793± 0.24€
10	ECP+EFV+EBS CR	3.121± 0.25€
11	ECP PR	3.116± 0.37€
12	EFV PR	3.473± 0.24¥
13	EBS PR	2.937± 0.53€
14	ECP+EFV PR	3.140± 0.31€
15	ECP+EBS PR	2.911± 0.29€
16	EFV+EBS PR	2.615± 0.17€
17	ECP+EFV+EBS PR	2.206± 0.09€

All Values are Mean □ S.E.M. (n=6); Significance values are (€) $P < 0.001$, (¥) $P < 0.01$ and (©) $P < 0.05$. Control group vs all groups by one way analysis variance test (ANOVA) followed by Dunnett's multiple comparison test.

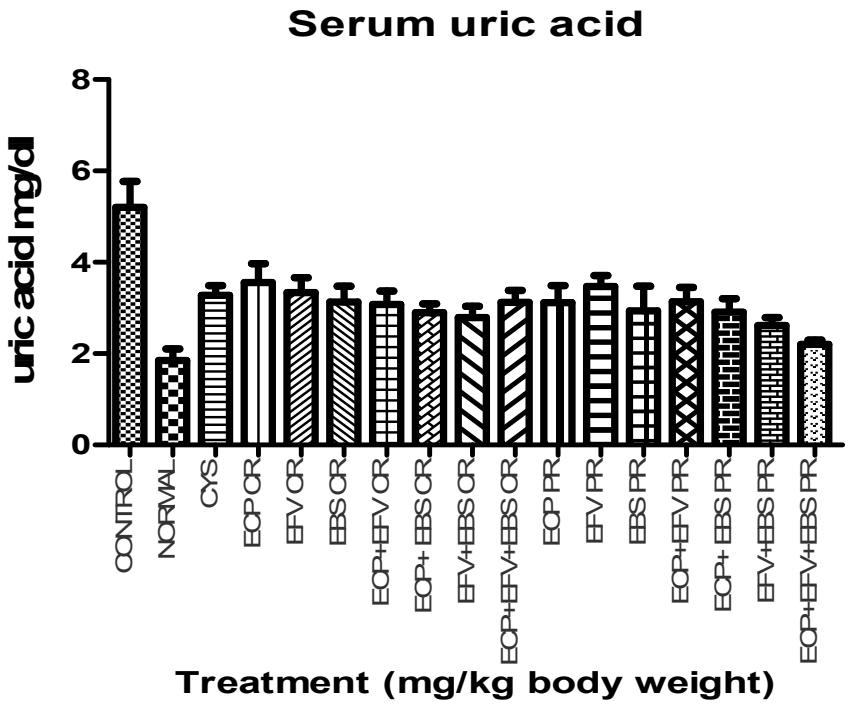


Figure 11. Graphical analysis of mean standard ± deviation ofuric acid

Table 12. Mean standard ± deviation of Serum Calcium (mg/dl)

Sr. No.	Sample Code	Serum Calcium (mg/dl)
1	Control	**23.29±0.63€**
2	Normal	**8.447±0.81€**
3	CYS	**9.818±0.99€**
4	ECP CR	**13.17±0.66€**
5	EFV CR	**12.49±0.48€**
6	EBS CR	**11.86±0.70€**
7	ECP+EFV CR	**13.37±0.59€**
8	ECP+EBS CR	**12.70±0.59€**
9	EFV+EBS CR	**11.14±0.69€**
10	ECP+EFV+EBS	**10.85±0.34€**

	CR	
11	ECP PR	**11.42±0.35**$^{€}$
12	EFV PR	**11.29±0.38**$^{€}$
13	EBS PR	**10.71±0.46**$^{€}$
14	ECP+EFV PR	**10.63±0.39**$^{€}$
15	ECP+EBS PR	**10.54±0.62**$^{€}$
16	EFV+EBS PR	**10.35±0.42**$^{€}$
17	ECP+EFV+EBS PR	**9.342±0.95**$^{€}$

All Values are Mean □ S.E.M. (n=6); Significance values are $^{(€)}$ $P < 0.001$, $^{(¥)}$ $P < 0.01$ and $^{(©)}$ $P < 0.05$. Control group vs all groups by one way analysis variance test (ANOVA) followed by Dunnett's multiple comparison test.

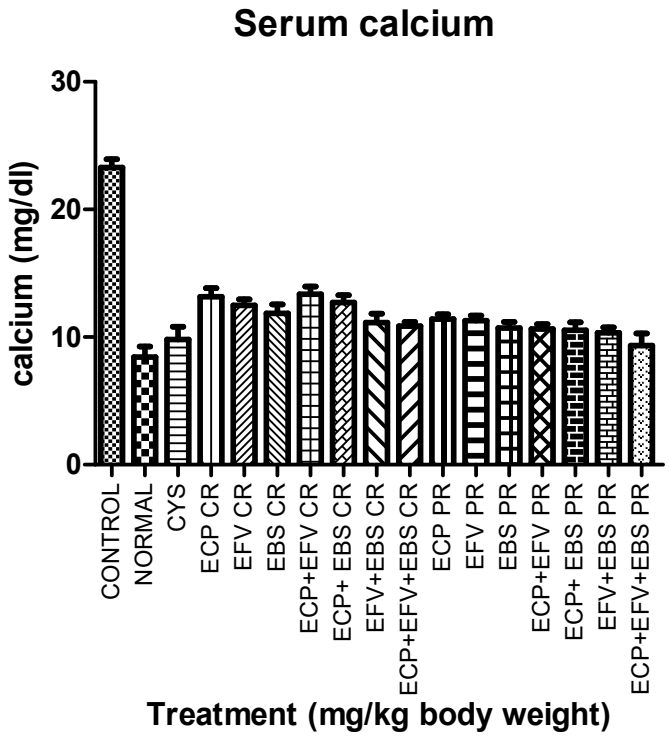


Figure 12. Graphical analysis of Mean standard ± deviation of serum calcium

Table 13. Mean standard ± deviation of Serum magnesium (mEq/L)

Sr. No.	Sample Code	Serum magnesium (mEq/L)
1	Control	1.037±0.1384$^{€}$
2	Normal	2.452±0.1335
3	CYS	1.758±0.1141$^{¥}$

4	ECP CR	1.679±0.1326[¥]
5	EFV CR	1.770±0.1015[¥]
6	EBS CR	1.670±0.04927[¥]
7	ECP+EFV CR	1.732±0.07503[¥]
8	ECP+EBS CR	1.770±0.06011[¥]
9	EFV+EBS CR	1.707±0.06941[¥]
10	ECP+EFV+EBS CR	1.788±0.05199[¥]
11	ECP PR	1.669±0.1632[€]
12	EFV PR	1.802±0.08171[¥]
13	EBS PR	1.906±0.08196[€]
14	ECP+EFV PR	1.862±0.06008[€]
15	ECP+EBS PR	1.927±0.1531[€]
16	EFV+EBS PR	2.020±0.1966[€]
17	ECP+EFV+EBS PR	2.355±0.2634[€]

All Values are Mean □ S.E.M. (n=6); Significance values are [(€)] $P < 0.001$, [(¥)] $P < 0.01$ and [(©)] $P < 0.05$. Control group vs all groups by one way analysis variance test (ANOVA) followed by Dunnett's multiple comparison test.

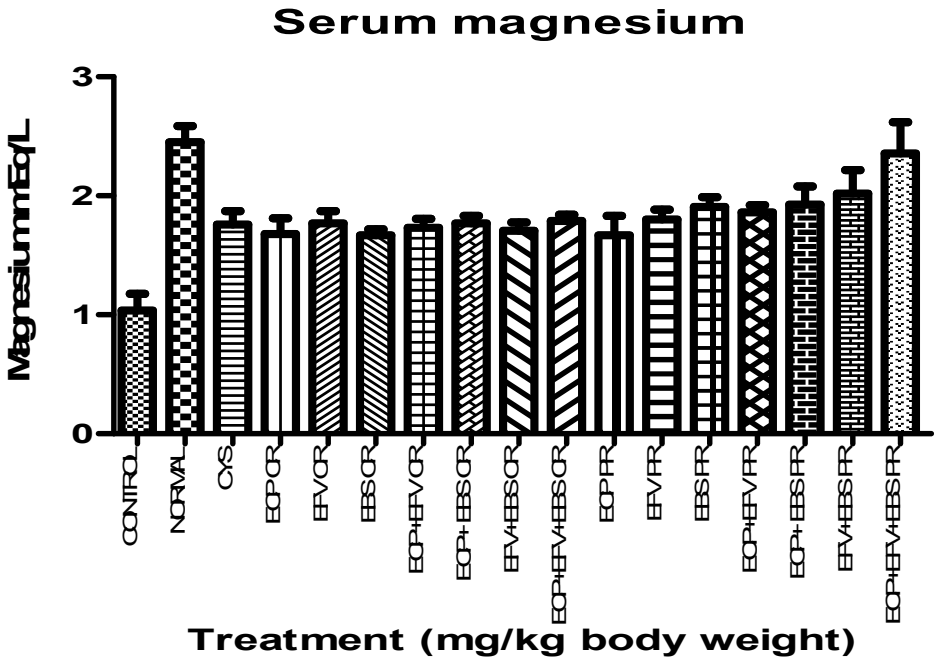


Figure 13. Graphical analysis of Mean standard ± deviation of serum magnesium

Renal stone induction caused impairment of renal functions of the untreated rats as evident from the markers of glomerular and tubular damage i.e elevated serum creatinine, uric acid , Serum phosphorus, uric acid and urea. These markers were significantly (P<0.05) reduced in the animals which were treated with CYS- Cystone, ECP CR- Ethanol extract of C*issampelos*

pareira L curative Regimen, EFV CR , Ethanol extract of Fragaria vesca L curative Regimen, BS CR- Ethanol extract of *Biophytum sensitivum L curative* Regimen *E*CP+EFV-CR - Ethanol extract of Combination of C*issampelos pareira* L and - *Fragaria vesca* Lcurative regimen, *E*CP+EBS CR- C*issampelos pareira* and *Biophytum sensitivum* L curative regimen, *E* FV+EBS CP- *Fragaria vesca L* and *Biophytum sensitivum* Curative Regimen ,ECP+EFV+EBS CR- C*issampelos pareira*, *Fragaria vesca* and *Biophytum sensitivum L curative* Regimen. ECP PR- Ethanol extract of C*issampelos pareira* L protective Regimen, EFV PR , Ethanol extract of Fragaria vesca protective Regimen, BS PR- Ethanol extract of *Biophytum sensitivum L* protectiveRegimenECP+EFV-PR - Ethanol extract of Combination of C*issampelos pareira* L and - *Fragaria vesca* L protective Regimen, *E*CP+EBS PR- C*issampelos pareira* and *Biophytum sensitivum* protective Regimen , *E* FV+EBS PR - *Fragaria vesca L* and *Biophytum sensitivum* protective Regimen. The combinationof ECP+EFV+EBS CRECP+EFV+EBS PR and C*issampelos pareira* L, *Fragaria vesca* Land *Biophytum sensitivum* L protectiveRegimen give best results as compare with standard cystone drug.

Table 14. Study of urine and serum parameter isolated fractions of C*issampelos pareira linn*

Isolated fraction 1	**Parameter (mg/dl).**	**Value**
Qurcetrine	Total Protein	7.602 ± 0.4270
	Urine Calcium	10.06 ±0.8534
	Urine Creatinine	9.900 ± 0.5455
	urine oxalate	14.300±0.5774
	urinary urea	482.5±18.95
	uric acid	9.272±0.4753
	Serum Creatinine	0.9200±0.03843
	Serum phosphorus,	1.423±0.1238
	serum urea	49.64±1.568
	Serum uric acid	4.381±0.2849

Table 15. Study of urine and serum parameter isolated fractions of Fragaria vesca linn

Isolated fraction 2	**Parameter (mg/dl).**	**Value**
Kaempferol	Total Protein	7.847 ±0.4604
	Urine Calcium	11.03± 0.5269
	Urine Creatinine	9.829±0.8415
	urine oxalate	13.33±0.5236
	urinary urea	438.4±23.54
	uric acid	9.809±0.4494
	Serum Creatinine	0.8458 ± 0.05286
	Serum phosphorus,	1.114±0.07802
	serum urea	46.55±1.643
	Serum uric acid	4.613±0.2945

Table 16. Study of urine and serum parameter isolated fractions of Biophytum Sensitivum linn

Isolated fraction 3	Parameter (mg/dl).	Value
Luteolin	Total Protein	7.942±0.3566
	Urine Calcium	9.564±0.8116
	Urine Creatinine	9.578±0.3000
	urine oxalate	13.25±0.9706
	urinary urea	339.8±15.32
	uric acid	9.180±0.3644
	Serum Creatinine	0.8630±0.01842
	Serum phosphorus,	1.137±0.1285
	serum urea	45.29±1.023
	Serum uric acid	4.338±0.3177

Table 17.Study of renal Calcium parameter isolated fractions of Extracts

Sr. No.	Sample Code	Renal Calcium (mg/dl)
1	Control	20.01± 1.15$^{€}$
2	Normal	4.40± 0.98$^{€}$
3	CYS	5.90± 0.79$^{€}$
4	ECP CR	11.30± 0.87$^{€}$
5	EFV CR	9.28± 1.04$^{€}$
6	EBS CR	7.67± 0.68$^{€}$
7	ECP+EFV CR	10.40± 0.58$^{€}$
8	ECP+EBS CR	10.54± 0.43$^{€}$
9	EFV+EBS CR	9.94± 0.53$^{€}$
10	ECP+EFV+EBS CR	8.90± 0.30$^{€}$
11	ECP PR	9.26± 0.57$^{€}$
12	EFV PR	9.23± 0.44$^{€}$
13	EBS PR	8.57± 0.32$^{€}$
14	ECP+EFV PR	9.91± 0.47$^{€}$
15	ECP+EBS PR	10.67± 1.09$^{€}$
16	EFV+EBS PR	7.26± 0.9235$^{€}$
17	ECP+EFV+EBS PR	6.87± 0.8361$^{€}$

All Values are Mean □ S.E.M. (n=6); Significance values are $^{(€)}$ $P < 0.001$, $^{(¥)}$ $P < 0.01$ and $^{(©)}$ $P < 0.05$. Control group vs all groups by one way analysis variance test (ANOVA) followed by Dunnett's multiple comparison test.

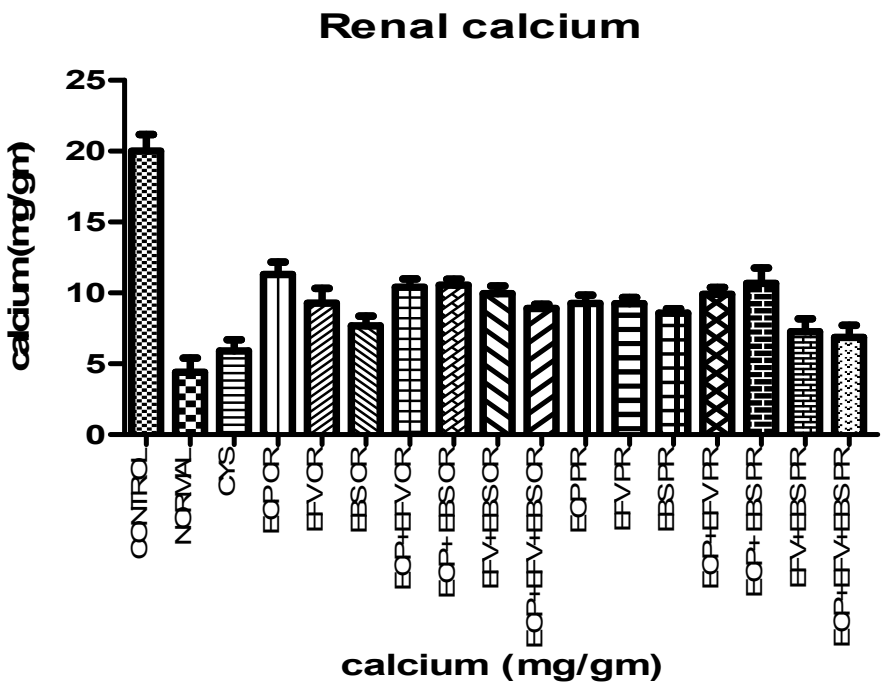


Figure 14. Study of renal Calcium parameter isolated fractions of Extracts

Table 18. Study of renal oxalate parameter isolated fractions of Extracts

Sr. No.	Sample Code	Renal oxalate (mg/dl)
1	Control	25.23±1.64€
2	Normal	8.912±0.81
3	CYS	9.400±1.08€
4	ECP CR	17.35±0.76€
5	EFV CR	11.73±0.91€
6	EBS CR	10.79±1.03€
7	ECP+EFV CR	9.743±0.53€
8	ECP+EBS CR	9.588±0.61€
9	EFV+EBS CR	8.890±0.49€
10	ECP+EFV+EBS CR	9.843±0.60€
11	ECP PR	15.80±2.20€
12	EFV PR	12.73±0.92€
13	EBS PR	10.19±1.34€
14	ECP+EFV PR	10.26±0.80€
15	ECP+EBS PR	10.28±0.47€
16	EFV+EBS PR	10.41±0.56€
17	ECP+EFV+EBS PR	9.573±0.52€

All Values are Mean □ S.E.M. (n=6); Significance values are (€) $P < 0.001$, (¥) $P < 0.01$ and (©) $P < 0.05$. Control group vs all groups by one way analysis variance test (ANOVA) followed by

Dunnett's multiple comparison test.

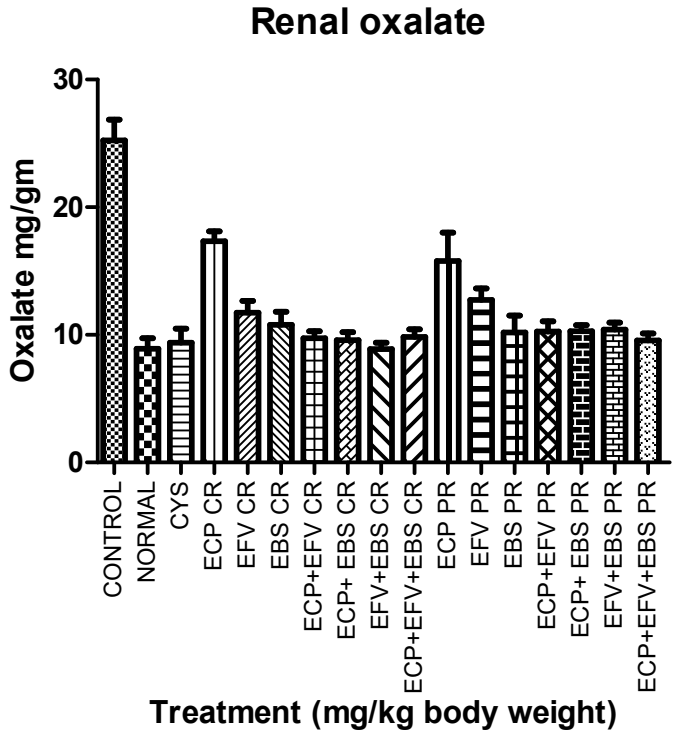
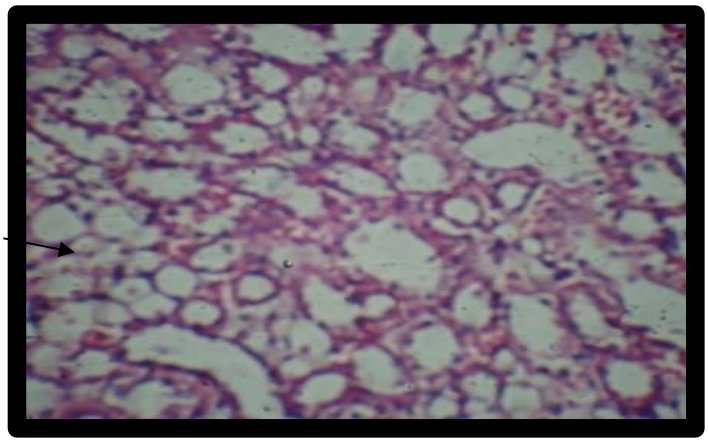


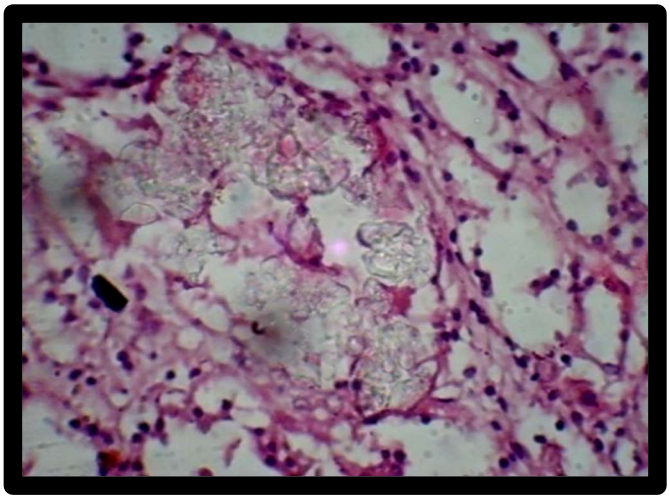
Figure 15. Study of renal oxalate parameter isolated fractions of extracts

Histopathology Studies

Ethylene glycolated water 0.75% (E. G. W) + AC 1% induced urolithiasis in rat.



A



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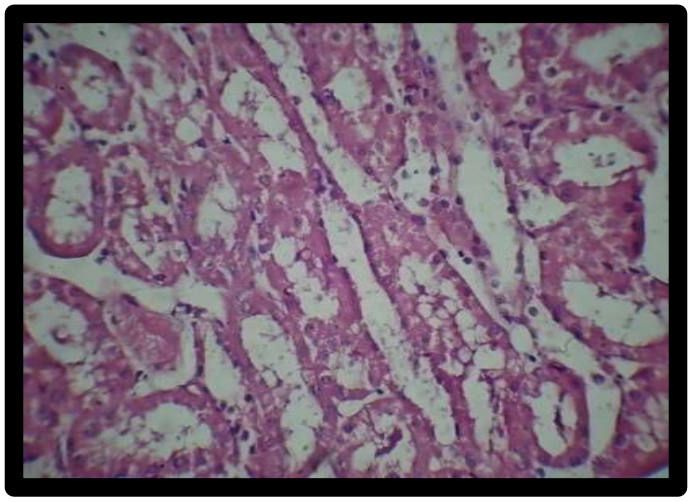
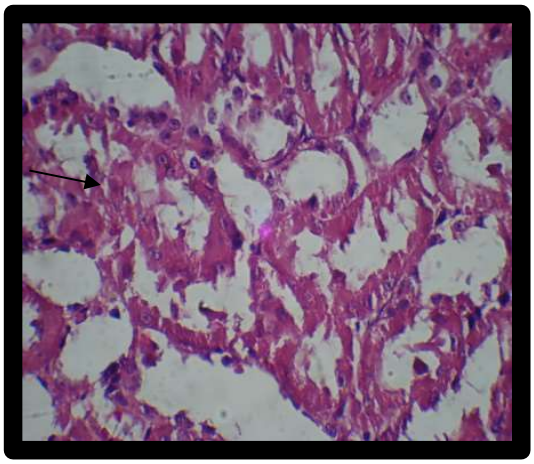


C

Figure 16. Histopathology studies of A=Normal, B= Control (Ethylene Glycolated Water), C= Standard (Cystone 750 mg/Kg) Hematoxylin and Eosin, x 40



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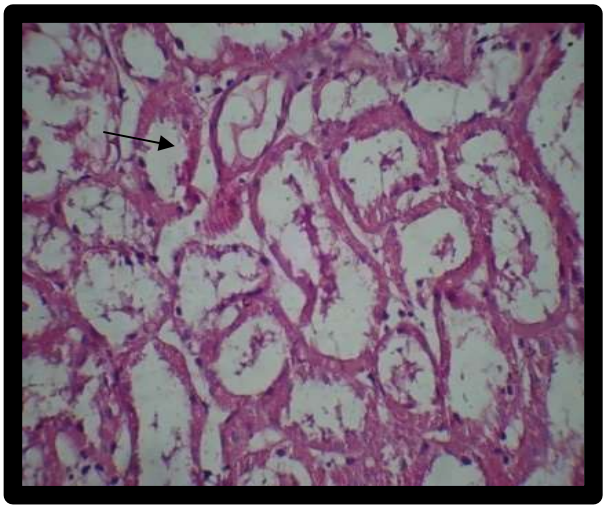

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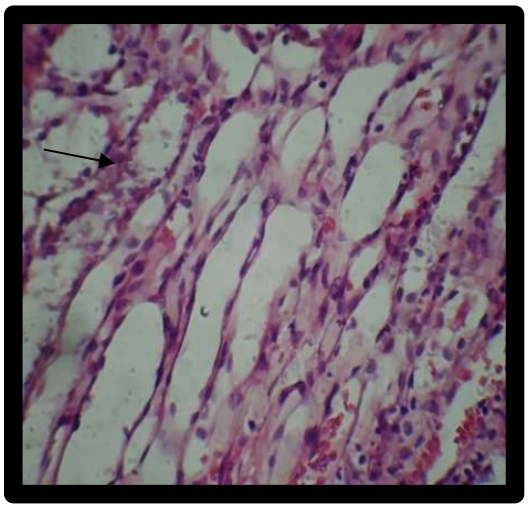

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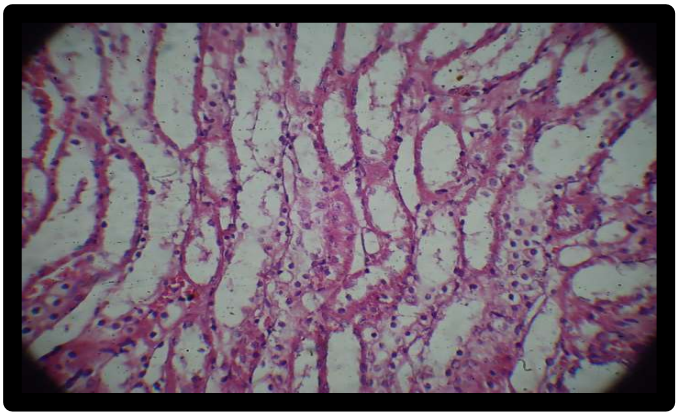

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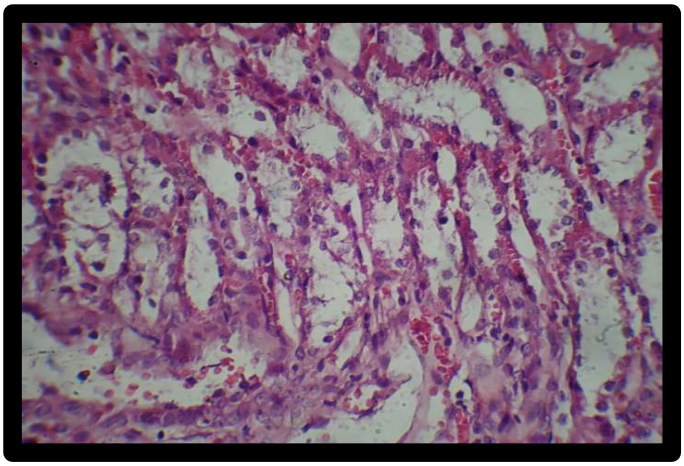

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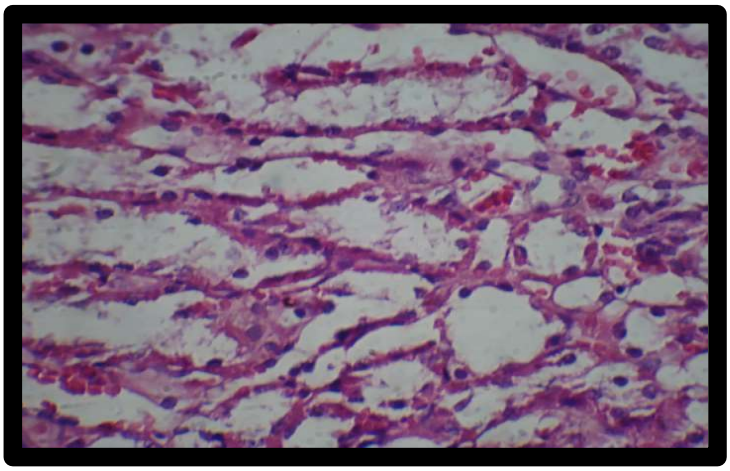

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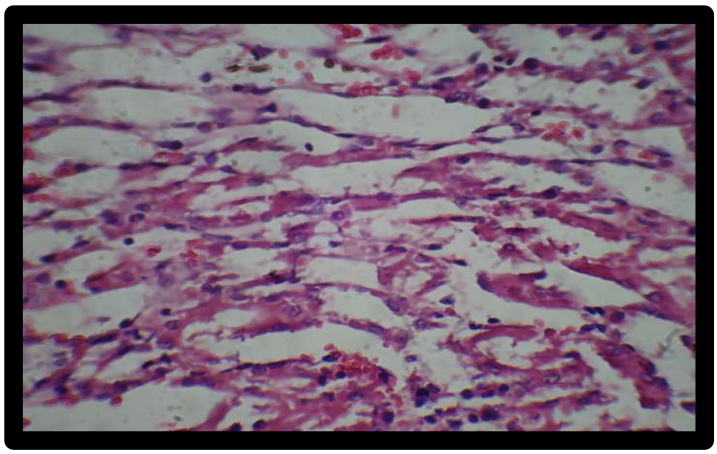

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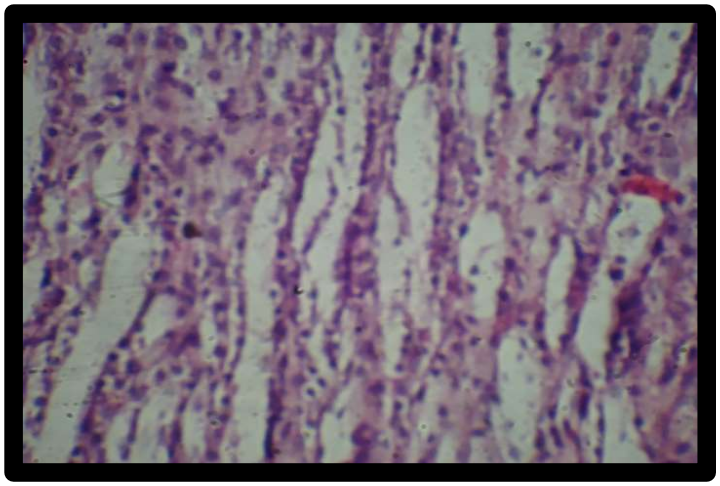

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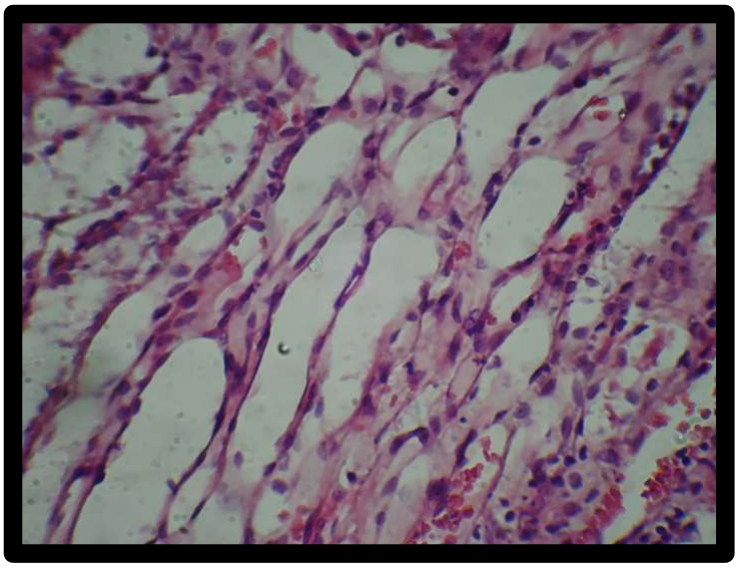

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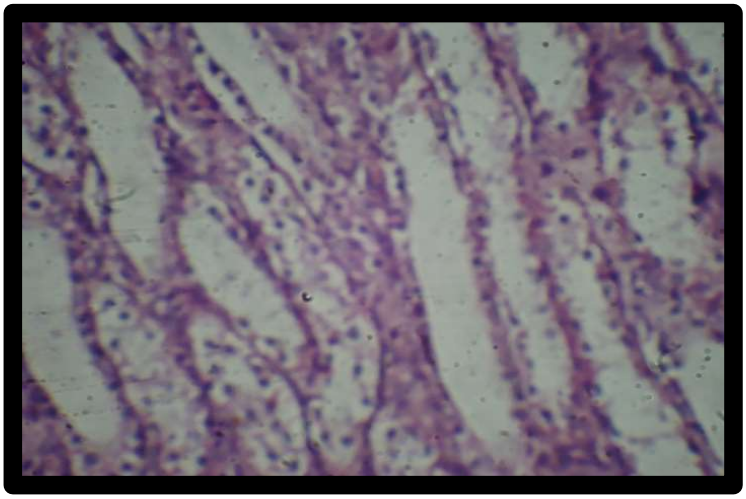

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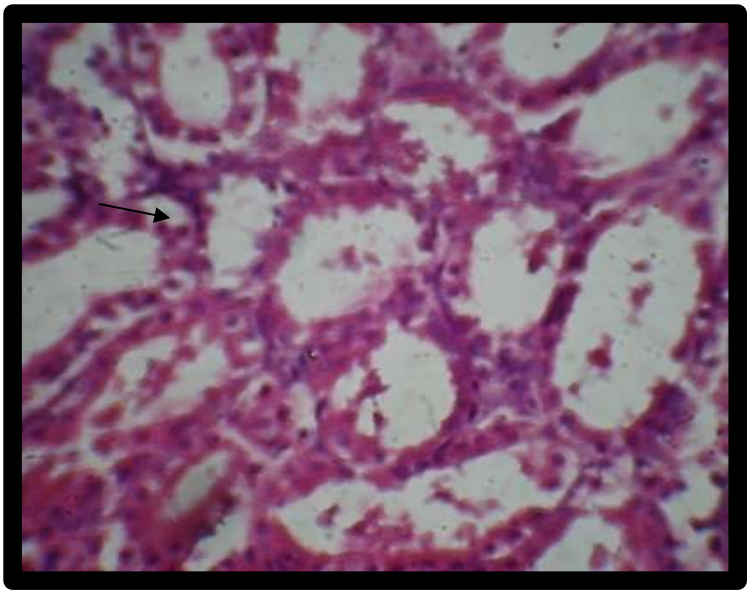

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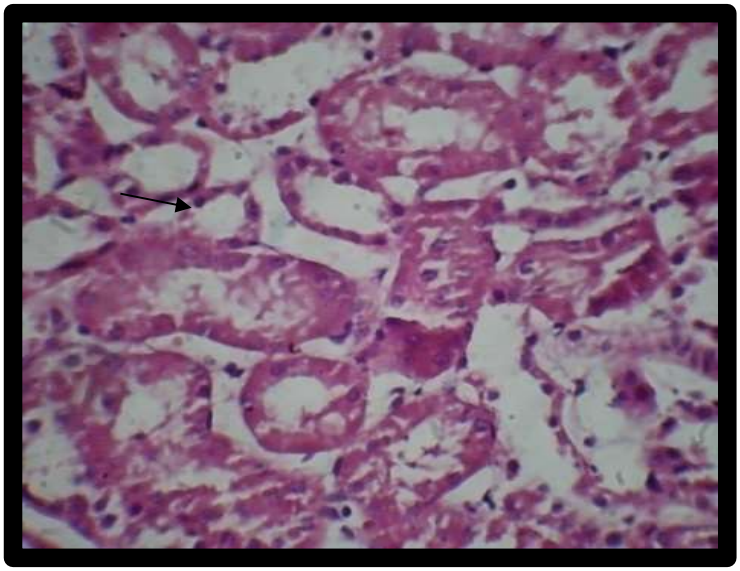

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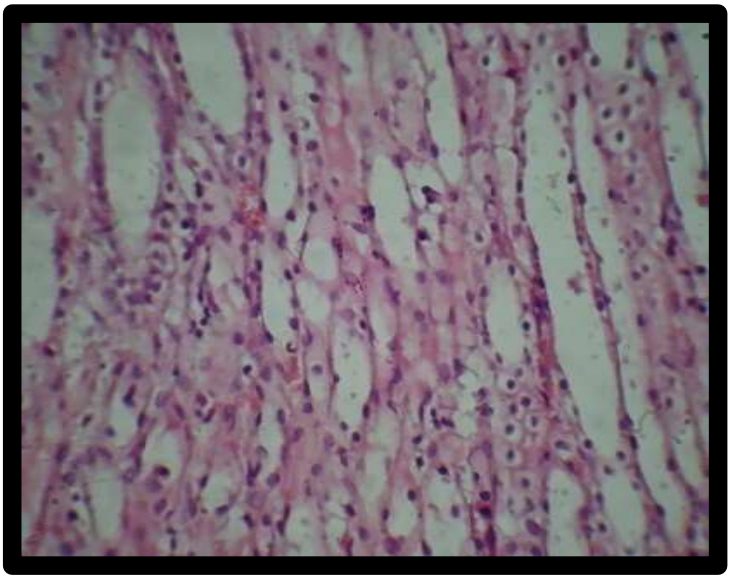

Q

Figure 17. Histopathology studies of extracts

D= **ECP CR**, E= **EFV CR**, F= **EBS CR**, G= ECP+EFV CR, H= ECP+ EBS CR, I= EFV+EBS CR, J= ECP+EFV+EBS CR, K= ECP PR L= EFV PR, M= EBS PR, N= ECP+EFV PR PR, O= . ECP+ EBS PR, P= EFV+EBS PR Q= ECP+EFV+EBS PR Hematoxylin and Eosin, x 40.

NORMAL:

Normal rats (Fig. A) show normal glomerular structure and renal tubules in normal.

CONTROL:

Conrtol E.G 0.75% induced urolithiasis (Fig. B round circal) shows crystal deposition abundant and visible bigger size in intratubular and lumen region. Renal tubular damage with epithelial damage, cystic atrophy of tubule and infiltration of lymphocyte with more tubular damage, dilation of tubuls.

TREATMENT:

Cystone 750 mg/kg

In the cystone treatment (fig. c thin arrow) no crystal deposition was seen, with little tutubular dilation and inflammation, regeneration of tubular epithelium cell.

ECP CR

In the GG 100 mg/kg treatment (fig. D thin arrow) no crystal deposition was seen with little degeneration of epithelial cell and tubular dilation as compared to control.

EFV CR

In the GG 200 mg/kg treatment (fig.E thin arrow) no crystal deposition was seen regeneration of epithelial cell and decreased inflammation as compared to control.

EBS CR

In the GG 200 mg/kg treatment (fig.F thin arrow) no crystal deposition was seen regeneration of epithelial cell and decreased inflammation as compared to control.

ECP+EFV CR

In the ECP+EFV CR treatment (fig. G thin arrow) no crystal deposition was seen with little degeneration of epithelial cell and tubular dilation as compared to control.

ECP+ EBS CR

In the = ECP+ EBS CR treatment (fig.H thin arrow) no crystal deposition was seen regeneration of epithelial cell and decreased inflammation as compared to control.

EFV+EBS CR

In the EFV+EBS CR treatment (fig.I thin arrow) no crystal deposition was seen regeneration of epithelial cell and decreased inflammation as compared to control.

ECP+ EBS CR

In the = ECP+ EBS CR treatment (fig.J thin arrow) no crystal deposition was seen regeneration of epithelial cell and decreased inflammation as compared to control.

ECP PR

In the ECP PR treatment (fig.K thin arrow) no crystal deposition was seen regeneration of epithelial cell and decreased inflammation as compared to control.

EFV PR

In the EFV PR treatment (fig.L thin arrow) no crystal deposition was seen regeneration of epithelial cell and decreased inflammation as compared to control.

EBS PR

In the EBS PR treatment (fig.M thin arrow) no crystal deposition was seen regeneration of epithelial cell and decreased inflammation as compared to control.

ECP+EFV PR

In the ECP+EFV PR treatment (fig.N thin arrow) no crystal deposition was seen regeneration of epithelial cell and decreased inflammation as compared to control.

ECP+ EBS PR

In the ECP+ EBS PR treatment (fig. O thin arrow) no crystal deposition was seen with little degeneration of epithelial cell and tubular dilation as compared to control.

EFV+EBS PR

In the EFV+EBS PR treatment (fig. P thin arrow) no crystal deposition was seen regeneration of epithelial cell and decreased inflammation as compared to control.

ECP+EFV+EBS PR

In the ECP+EFV+EBS PR treatment (fig. Q thin arrow) no crystal deposition was seen regeneration of epithelial cell and decreased inflammation as compared to control.

3. Conclusion

All the extracts were studied for in-vitro activity with the nucleation assay, Growth Assay, Cell Culture, Cytotoxicity - Trypan Blue Assay, LDH Leakage Assay for effective inhibition of crystal growth. The combination of extract shows better results for inhibition of growth. On this basis, it was selected for further studies. All the extracts were studied for acute oral toxicity study using OECD guidelines 423. All alcoholic extract of cissampelous perrira linn, fragarica vesca linn , biophytum sensitivum linn shows Preliminary oral Lethal Dose values were found to be 2000 mg/kg and 5000 mg/kg . 1/10th of this LD_{50} was taken as effective dose (therapeutic dose) for subsequent studies. In the course of our studies Experimental design for 75% EGW +

AC 1% models for inducing kidney stone in the rats.Different evaluation Parameter for anti-urolithiatic models were carried out such asbiochemical Parameters in serum like creatinine, uric acid, urea, calcium, phosphorus and magnesium and citrate. and Parameters in urine like urea, creatinine, uric acid, magnesium, citrate, calcium, phosphorus, oxalate and pH., effect of extract was compared with Standard reference compound cystone. In the present study shows that an increased in urine output of extract of*Cissampelos pareira*, *fragarica vesca* and *Biophytum sensitivum* treated animals which dilute the concentration of urinary electrolytes. As a result, **calcium and phousphouraus** are flused out via the urine and there are lesser chances of precipitation, decreased formation as well as the growth of urinary stone. The excretion of **oxalate and calcium** were progressively increased in calculi induced animals. Also deposition of the crystalline components in the renal tissue, namely oxalate, phosphate, and calcium, were increased in the stone forming rat.Ethanol extract of C*issampelos pareira*, *fragarica vesca* and *Biophytum sensitivum*significantly ($P < 0.001$) lowered the elevated levels of oxalate, calcium and phosphate in urine and in CR and PR as compared to calculi-induced animals.

The glomerular filtration rate decreased in urolithiasis due to obstruction to the outflow of urine by stones in the urinary system and the west product such as **urea and uric acid** get accumulated. This indicates marked damage of kidney. The **uric acid** crystals adsorb glutamic acid and other organic compounds and promote calcium oxalate crystal growth. The results showed that a significant increase in uric acid level in serum as well as in urine in the ethylene glycol control group to normal control. The uric acid levels decreased after treatment with extract of C*issampelos pareira*, *Fragarica vesca* and *Biophytum* sensitivum and cystone, therefore hastening the process of dissolving the preformed stone and prevention of new stone formation in urinary system. Renal function was evaluated by measuring serum phosphorus, calcium, urea, and creatinine in Group I–XVII.The concentration of phosphorus, calcium, urea, and creatinine in the serum was significantly ($P< 0.001$ vs. Group I) increased in the stone-induced group indicating renal damage. However, treatment with *The combination of* ECP+EFV+EBS CRECP+EFV+EBS PR and C*issampelos pareira*, *fragarica vesca* and *Biophytum sensitivum* protectiveRegimen give best results as compare with standard cystone drug significantly ($P < 0.001$) reduced the concentrations of phosphorus, calcium, urea, and creatinine in the serum in both the prophylactic and curative groups to a near-normal level and were comparable to the standard group. The results indicate that administration of extracts and inhibit the growth of urinary stones. It is also seen that the prophylactic effect is more efficient than the curative effect. Therefore, the extracts is useful to prevent the recurrence of urolithiasis as it proved its effect on the early stages of stone development. related to increased diuresis and lowering of urinary concentrations of stone-forming components.

From the above result it can be concluded that-

Extraction of selected plant i.e species C*issampelos pareira L*, *fragarica vesca L* and *Biophytum sensitivum* L was carried out by using various solvents. And ethanol shows best results in %yield.so ethanol is choice of solvent for further isolation process.

Anti-urolithiasis activity was Evaluated with the abilities of bioactive agents with plant extracts used for urolithiasis in-vivo with animal models can be served as an aid in the evaluation of

novel treatments for urolithiasis.Protective nature of isolate and extract Reveals symptoms of urinary calculi like pain, burning micturition and haematuria.

1) Evaluate the abilities of bioactive agents with plant extracts used for urolithiasis in animal models served as an aid in the evaluation of novel treatments for urolithiasis.

2) With of protective action may be one way forward in minimizing tissue injury in human disease.

4. References

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