



## EFFECT OF FRUIT OF CUMINUM CYMINUM ON ETHYLENE GLYCOL (EG) INDUCED UROLITHIASIS IN RATS.

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### ABSTRACT

To evaluate the antiurolithiatic effect of the methanolic extracts of the fruit of *c. cyminum* on ethylene glycol (EG) induced urolithiasis in rats. To evaluate urinary excretion of calcium and phosphorous in normal and induced groups. To evaluate urinary excretion of calcium and phosphorous in methanolic extract treated groups. Collection and Authentication of plant. Preparation of extract of seeds using 95%v/v methanol as a solvent. Albino rats (wistar strain), weighing 150-200g, were procured from the Teena biolabs pvt. Ltd. (Reg. no. 177/99 CPCSEA). Rangareddy, Andhrapradesh. The animals were kept in polypropylene cages (6 in each cage) and free access to commercial pellet diet with water ad libitum. Methanolic extract of *c. cyminum* Linn, significantly reduced the elevated levels of calcium, phosphorous, blood urea nitrogen, uric acid & serum creatinine in curative & preventive treatment groups. The histopathological findings also show sign of improvement after treatment with the methanolic extract of *c. cyminum*. All these observation provided that the basis for the conclusion that *c. cyminum* Linn seed extract inhibited the stone formation induced by ethylene glycol treatment.

**KEYWORDS:** Antiurolithiatic, methanolic, *c. cyminum*.

### AIM AND OBJECTIVES

- To evaluate the antiurolithiatic effect of the methanolic extracts of the fruit of *c. cyminum* on ethylene glycol (EG) induced urolithiasis in rats.
- To evaluate urinary excretion of calcium and phosphorous in normal and induced groups.
- To evaluate urinary excretion of calcium and phosphorous in methanolic extract treated groups.
- To evaluate serum parameters like Blood urea nitrogen, Serum creatinine and Uric acid in normal and induced groups.
- To evaluate serum parameters like Blood urea nitrogen, Serum creatinine and Uric acid in methanolic extract treated groups.
- To find out the histopathology of rat kidneys in normal, induced and methanolic extract treated groups.

### 5. PLAN OF WORK

The following studies were done on the seed extract of *c. cyminum* Linn.

- ❖ Collection and Authentication of plant.
- ❖ Preparation of extract of seeds using 95%v/v methanol as a solvent.
- ❖ Preliminary phytochemical analysis using the whole plant extract.
- ❖ To evaluate acute oral toxicity studies of methanolic extract of *c. cyminum*.

- ❖ Induction of urolithiasis.
- ❖ To study antiurolithiatic activity of methanolic extract of *c. cyminum*.
- ❖ Evaluation of urinary parameters.
  - Estimation of calcium.
  - Estimation of phosphorous.
- ❖ Evaluation of serum parameters.
  - Estimation of Blood urea nitrogen.
  - Estimation of Serum creatinine.
  - Estimation of Uric acid.
- ❖ Estimation of kidney weights.
- ❖ Histopathological studies of rat kidneys.
- ❖ Statistical analysis of the results.

### 5.1 Plant material

The seeds of *c. cyminum* were collected from local market of Karimnagar, Andhrapradesh, India. It will be taken authentication from the department of Botany, Kakatiya University.

### Preparation of extract

- The seeds of *c. cyminum* were reduced to coarse powder by mechanical grinding. The powdered material 100 g was subjected to continue hot extraction in soxhlet by using methanol (95% v/v). The extraction was continued until the solvent in the thimble became clear.

- After complete extraction, the extract was dried. The extract was stored in deep freezer at 20°C for the experimental use.
- The extract was solubilised in distilled water and used for studying the antiurolithiatic activity (Khatib Nayeem et al., 2000).

### 5.2 phytochemical screening of *c. cyminum*

The phytochemical screening of the methanolic extract of *c. cyminum* was carried out to detect steroids and flavanoids (Kokate CK 1994).

### Test for flavonoids

To the small quantity of ethanolic residue add few lead acetate solution. Yellow colour precipitate is developed, shows the presence of flavanoids.

### Test for steroids

Add small quantity of extract with chloroform. Add 2ml of acetic anhydride and 2 drops of concentrated sulphuric acid from the side of test tube. First red, blue then finally green colour appears.

## 5.3 MATERIALS AND METHODS

**Table 2: Chemicals with source**

| S.No | CHEMICALS               | SOURCE                                            |
|------|-------------------------|---------------------------------------------------|
| 1    | Acasia                  | Universal laboratories pvt. ltd. Mumbai, India.   |
| 2    | Blood urea nitrogen Kit | M/S Excel diagnostics pvt. ltd. Hyderabad, India. |
| 3    | Calcium Kit             | M/S Excel diagnostics pvt. ltd. Hyderabad, India. |
| 4    | Chloroform              | S D Fine chemicals, Mumbai, India.                |
| 5    | Cystone                 | The Himalaya drug co. Bangalore, India.           |
| 6    | Ethylene glycol         | S D Fine chemicals, Mumbai, India.                |
| 7    | Formaldehyde            | S D Fine chemicals, Mumbai, India.                |
| 8    | Phosphorous Kit         | M/S Excel diagnostics pvt. ltd. Hyderabad, India. |
| 9    | Serum creatinine Kit    | M/S Excel diagnostics pvt. ltd. Hyderabad, India. |
| 10   | Uric acid Kit           | M/S Excel diagnostics pvt. ltd. Hyderabad, India. |

### 5.4 Experimental design

Albino rats (wistar strain), weighing 150-200g, were procured from the Teena biolabs pvt. Ltd. (Reg. no. 177/99 CPCSEA). Rangareddy, Andhrapradesh. The animals were kept in polypropylene cages (6 in each cage) and free access to commercial pellet diet with water *ad libitum*. Animals were kept in animal house of SRR College of Pharmaceutical Sciences-Karimnagar. The animal house temperature was maintained at 25 ± 2°C with relative humidity at (50 ± 15%) and light: dark exposure of 12:12 h. The experiments were carried out prior approval from Institutional Animal Ethical Committee (IAEC) approval no. (1322/ac/10/CPCSEA). Ethical norms were strictly followed during the experiment.

### 5.5 Acute toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines. Albino mice (n = 3) of either sex selected by random sampling technique were used for acute toxicity study. The animals were kept fasting overnight providing only water, after which the extract (50% alcoholic extract) was administered orally at the dose

level of 5 mg/kg body weight by gastric intubation and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose would be repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg/kg body weight. According to the results of the acute toxicity test, the doses were chosen for experiments.

### 5.6 Gross behavioural changes

The animals were observed for behavioural, neurological and autonomic profiles during acute toxicity studies (Sheth et al., 1972).

### 5.7 Experimental procedure: Antiurolithiatic study

#### Induction of urolithiasis by ethylene glycol in rats

Lithiasis was induced by administering by gastric intubation of 0.75% ethylene glycolated water to the animals except control animals up to 28 days. The control animals received vehicle.

**Table 3: Protocol for antiurolithiatic activity.**

| S.No | Group | Treatment                                                                    | Route of administration | Duration                                    |
|------|-------|------------------------------------------------------------------------------|-------------------------|---------------------------------------------|
| 1    | I     | Control group with water                                                     | oral                    | 28 days                                     |
| 2    | II    | Ethylene glycol 0.75% + water                                                | oral                    | 28 days                                     |
| 3    | III   | Ethylene glycol 0.75% + Cystone 750 mg/kg b.w.                               | oral                    | 15 <sup>th</sup> day to 28 day              |
| 4    | IV    | Ethylene glycol 0.75% + methanolic extract of <i>C.cyminum</i> 200mg/kg b.w. | oral                    | 15 <sup>th</sup> day to 28 day              |
| 5    | V     | Ethylene glycol 0.75% + methanolic extract of <i>C.cyminum</i> 400mg/kg b.w. | oral                    | 15 <sup>th</sup> day to 28 day              |
| 6    | VI    | Ethylene glycol 0.75% + methanolic extract of <i>C.cyminum</i> 200mg/kg b.w. | oral                    | 1 <sup>st</sup> day to 28 <sup>th</sup> day |
| 7    | VII   | Ethylene glycol 0.75% + methanolic extract of <i>C.cyminum</i> 400mg/kg b.w. | oral                    | 1 <sup>st</sup> day to 28 <sup>th</sup> day |

### Experimental Procedure

Animals were divided into seven groups, each containing six animals. Group I served as normal control and treated with 1% acasia solution per oral route. Group II to VII were fed with 0.75% ethylene glycol (EG) in water for induction of renal calculi till 28th day (p.o). Group III received standard antiurolithiatic drug cystone (750 mg/kg body weight) from 15th to 28th day (p.o). Group IV served as curative regimen, received methanolic extract of the fruit of *C. cyminum* at a dose of 200 mg/kg body weight from 15th day to 28th day respectively (p.o). Group V served as curative regimen, received methanolic extract of the fruit of *C. cyminum* at a dose of 400 mg/kg body weight from 15th day to 28th day respectively (p.o). Groups VI served as preventive regimen received methanolic extract of the fruit of *C. cyminum* at a dose of 200 mg/kg body weight from 1st day to 28th day respectively (p.o). Groups VII served as preventive regimen received methanolic extract of the fruit of *C. cyminum* at a dose of 400 mg/kg body weight from 1st day to 28th day respectively (p.o) (Purnima Ashok et al., 2010).

### 5.8 Collection & analysis of urine samples

- Urine samples were collected on 28th day for 24 h by keeping the animals in individual propylene metabolic cages.

### Procedure

To three clean, dry tubes labeled Blank (B), Standard (S), Test (T), added the reagents in the following order.

| Reagents         | Blank (ml) | Standard (ml) | Test (ml) |
|------------------|------------|---------------|-----------|
| Buffer reagent   | 0.5        | 0.5           | 0.5       |
| Colour reagent   | 0.5        | 0.5           | 0.5       |
| Distilled water  | 0.02       | -             | -         |
| Calcium standard | -          | 0.02          | -         |
| Sample           | -          | -             | 0.02      |

Mix well and incubate at R. T. (25°C) for 5 minutes. Measure the absorbance of the Standard (Abs. S) and Test Sample (Abs. T) at 570 nm against Blank, within 60 minutes.

- Animals had free access to drinking water during the urine collection period. The collected urine was analyzed for calcium and phosphorous using standard methods.

### ESTIMATION OF URINE PARAMETERS

#### 5.8.1 DETERMINATION OF CALCIUM: (OCPC Method)

Calcium in an alkaline medium combines with o-Cresolphthalein Complexone to form a purple coloured complex. Intensity of the colour formed is directly proportional to the amount of calcium present in the sample.

Calcium + OCPC → Purple Coloured Complex

#### Reagents

1. Buffer Reagent
2. Colour Reagent
3. Calcium Standard (10 mg/dl).

**Sample:** urine.

#### Calculation

$$\text{Calcium in mg/dl} = \frac{\text{Abs of test}}{\text{Abs of std}} \times 10.$$

### 5.8.2 DETERMINATION OF PHOSPHOROUS: (Modified metol method)

Ammonium molybdate under acidic conditions reacts with phosphorus to form phosphomolybdate complex which is reduced to blue colored complex by metol. The absorbance of color developed is proportional to the inorganic phosphorus concentration.



#### Reagents

1. Acid reagent
2. Molybdate reagent
3. Colour reagent
4. Phosphorus standard (5mg/dl).

**Sample:** urine.

#### Procedure

To three clean, dry tubes labeled Blank (B), Standard (S), Test (T), added the reagents in the following order.

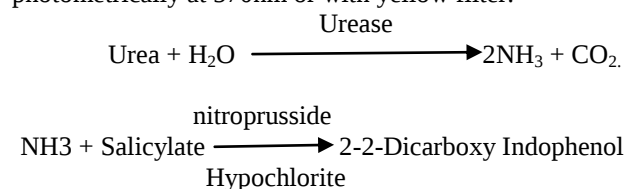
| Reagents                      | Blank (ml) | Standard (ml) | Test (ml) |
|-------------------------------|------------|---------------|-----------|
| Catalyst reagent              | 1.0        | 1.0           | 1.0       |
| Molybdate reagent             | 1.0        | 1.0           | 1.0       |
| Metol reagent                 | 1.0        | 1.0           | 1.0       |
| Deionized water               | 0.1        | -             | -         |
| Phosphorus standard (5mg/dl). | -          | 0.1           | -         |
| Sample                        | -          | -             | 0.1       |

Mix well and incubate at room temperature for five minutes. Measure the absorbance of Standard(s) and Test(T) against Blank (B) either on a photocolrimer with red filter or on Spectrophotometer at 680 nm, within 30 minutes.

#### Calculation

$$\text{Phosphorous in mg\%} = \frac{\text{Abs of test}}{\text{Abs of std}} \times 0.5.$$

mixture of salicylate, hypochlorite and Nitroprusside to yield a blue-green colored compound (Indophenol). The intensity of color produced is proportional to the concentration of urea in the Sample and is measured photometrically at 570nm or with yellow filter.



### 5.9 Serum analysis

- After the experimental period, rats were anaesthetized with ether and blood was collected from the retro orbital puncture.
- Serum was separated by centrifugation at 10,000 rpm for 10 minutes and analyzed for creatinine, uric acid and BUN.

#### Reagents

1. Urease Reagent
2. Colour Reagent
3. Urea Standard (40 mg%)
4. **Sample:** Serum

### ESTIMATION OF SERUM PARAMETERS

#### 5.9.1 DETERMINATION OF BLOOD UREA NITROGEN: (Berthelot method)

Urease catalyses the conversion of Urea to Ammonia and Carbondioxide. The Ammonia released reacts with a

#### Procedure

To three clean, dry tubes labeled Blank (B) Stand (S) and Test (T), added the reagents in the following order.

| Reagents       | Blank (ml) | Standard(ml) | Test (ml) |
|----------------|------------|--------------|-----------|
| Urease reagent | 1.0        | 1.0          | 1.0       |
| Urea Standard  | -          | 0.01         | -         |
| Serum          | -          | -            | 0.01      |
| Colour reagent | 1.0        | 1.0          | 1.0       |

Mix and incubate for 5 min at 37°C/10 min. at RT & read absorbance of standard (S) and Test (T) against Blank (B) at 570nm or with yellow filter. The final color is stable for 30 min at R.T.

#### CALCULATIONS

$$\text{Urea in mg\%} = \frac{\text{Abs. of (T)}}{\text{Abs. of (S)}} \times 40 (\text{Std. Conc.})$$

**Urea Nitrogen mg % = Urea (mg %) X 0.467**

### 5.9.2 DETERMINATION OF SERUM CREATININE: (Alkaline picrate method)

Picric acid in an alkaline medium reacts with creatinine to form an orange coloured complex with the alkaline picrate. Intensity of the colour formed is directly proportional to the amount of creatinine present in the sample.

Creatinine+Alkaline Picrate  $\longrightarrow$  Orange Coloured Complex.

#### Reagents

1. Picric acid Reagent
2. Buffer reagent

#### Step II: Color development

Labelled three clean and dry test tubes as Blank (B), Standard (S) and Test (T) and the following reagents were added:

| Reagents                | Blank (ml) | Std (ml) | Test (ml) |
|-------------------------|------------|----------|-----------|
| Supernatant from step-I | -          | -        | 1.1       |
| Creatinine Standard     | -          | 0.1      | -         |
| Distilled water         | 0.1        | -        | -         |
| Picric acid reagent (1) | 1.0        | 1.0      | -         |
| Buffer reagent (2)      | 0.1        | 0.1      | 0.1       |

Mixed well after each addition and allowed to stand at room temperature for exactly 20 minutes and measured the absorbance of blank (B), standard (S) and test (T) against distilled water on spectrophotometer at 520 nm.

#### CALCULATION

$$\text{Serum creatinine in mg\%} = \frac{\text{Abs of test}}{\text{Abs of standard}} \times 2.0 \text{ (std. conc.)}$$

#### PROCEDURE

| Reagents        | Blank (ml) | Standard (ml) | Test (ml) |
|-----------------|------------|---------------|-----------|
| Enzyme reagent  | 1.0        | 1.0           | 1.0       |
| Distilled water | 0.1        | -             | -         |
| Standard        | -          | 0.1           | -         |
| Sample          | -          | -             | 0.1       |

Mix well and incubate at 37°C for 10 min. Measure the absorbance of Test (T) and Standard (S), against Blank (B) on a colorimeter or spectrophotometer at 540 nm.

#### CALCULATION

$$\text{Uric acid in mg/dl} = \frac{\text{Abs of test}}{\text{Abs of standard}} \times 10 \text{ (std conc.)}$$

### 5.10 HISTOPATHOLOGICAL STUDIES

Two animals kidneys from each group were used for histological studies. The isolated kidneys were fixed in 10% neutral buffered formalin and then placed in fresh fixative solution and fixed for 1 week. Kidneys coronally cut and half of each kidney selected for pathological examination was processed for paraffin wax embedding. Four  $\mu$ m thick, sagittal slides from each kidney were taken from each block. Four sections were cut and

3. Standard (2mg /dl)

#### Step I: Deproteinization of specimen

pipette into a clean dry test tube labeled (T).

| Reagents                | Test (ml) |
|-------------------------|-----------|
| Serum                   | 1.0       |
| Picric acid reagent (1) | 6.0       |

Mixed well after each addition and filtered through Whatmann no.1 filter paper centrifuged at 2000-3000 rpm for 10 min to obtain a clear filtrate/supernatant.

### 5.9.3 DETERMINATION OF URIC ACID

Uric Acid is the major product of the catabolism of endogenous & exogenous(dietary) purine nucleosides (adenosine & guanosine). This transformation mainly occurs in the liver. Approximately 75% of Uric Acid is eliminated by kidneys, the remainder is secreted into the gastrointestinal tract, where it is degraded by bacterial enzymes.

stained with hematoxylin eosin for light microscopy using a conventional protocol. A pathologist, blinded to sample identity, examined all samples using light microscopy.

#### 5.11 Statistical Analysis

Results are expressed as mean  $\pm$  SEM. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison test. Differences between the data were considered significant at  $P < 0.05$ .

## 6. RESULTS

### PHARMACOLOGICAL STUDIES

#### Acute toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines.  $1/10^{\text{th}}$  of LD50 was taken as a therapeutic dose.

### Gross behavioral changes

The animals did not show any gross behavioral changes except for an increase in urination.

### Antiurolithiatic study

#### Urinary excretion of calcium and phosphorous

Urinary excretion of calcium was reduced after 28 days of treatment with *c.cyminum* treated preventive & curative groups ( $17.59 \pm 0.13$ ,  $16.12 \pm 0.12$ ,  $15.11 \pm 0.13$ ,  $13.58 \pm 0.17$ ) and standard treatment group ( $12.77 \pm 0.18$ ) when compared with the ethylene glycol treated group ( $22.02 \pm 0.36$ ). The elevated levels of calcium in methanolic extract treated groups are controlled equal to normal group values are represented in table 1 and figure 1.

Similarly urinary excretion of phosphorous was reduced after 28 days of treatment with *c.cyminum* treated preventive & curative groups ( $2.477 \pm 0.01$ ,  $2.323 \pm 0.01$ ,  $2.025 \pm 0.02$ ,  $1.723 \pm 0.01$ ) and standard treatment group ( $1.520 \pm 0.01$ ) when compared with the ethylene glycol treated group ( $2.938 \pm 0.05$ ). The elevated levels of calcium in methanolic extract treated groups are controlled equal to normal group values are represented in table 1 and figure 1.

#### Kidney weights

Increased kidney weight was reduced after 28 days of treatment with *c.cyminum* treated preventive & curative groups ( $0.81 \pm 0.011$ ,  $0.78 \pm 0.008$ ,  $0.74 \pm 0.005$ ,  $0.70 \pm 0.008$ ) and standard treatment group ( $0.67 \pm 0.005$ ) when compared with the ethylene glycol treated group ( $0.89 \pm 0.32$ ). Increased kidney weight in methanolic extract treated groups are controlled equal to normal group, values are represented in table 1 figure 1.

### Serum analysis

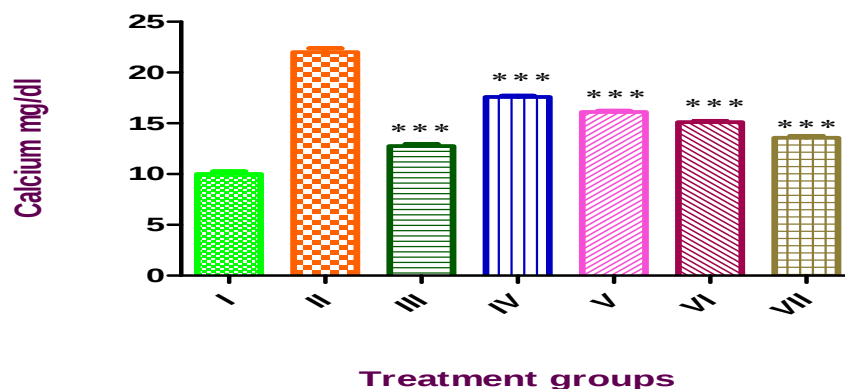
Increased levels of uric acid was reduced after 28 days of treatment with *c.cyminum* treated preventive & curative groups ( $5.613 \pm 0.14$ ,  $5.367 \pm 0.278$ ,  $4.700 \pm 0.12$ ,  $3.132 \pm 0.182$ ) and standard treatment group ( $4.797 \pm 0.13$ ) when compared with the ethylene glycol treated group ( $5.812 \pm 0.09$ ). Increased levels of uric acid in methanolic extract treated groups are controlled equal to normal group values are represented in table 1 and figure 1.

Similarly increased levels of blood urea nitrogen was reduced after 28 days of treatment with *c.cyminum* treated preventive & curative groups ( $34.55 \pm 2.32$ ,  $25.01 \pm 0.42$ ,  $28.61 \pm 1.24$ ,  $21.76 \pm 0.36$ ) and standard treatment group ( $23.68 \pm 0.41$ ) when compared with the ethylene glycol treated group ( $40.47 \pm 1.70$ ). Increased levels of blood urea nitrogen in methanolic extract treated groups are controlled equal to normal group values are represented in table 1 and figure 1.

Similarly increased levels of serum creatinine was reduced after 28 days of treatment with *c.cyminum* treated preventive & curative groups ( $5.033 \pm 0.20$ ,  $3.333 \pm 0.98$ ,  $3.800 \pm 0.23$ ,  $2.633 \pm 0.09$ ) and standard treatment group ( $2.867 \pm 0.13$ ) when compared with the ethylene glycol treated group ( $5.433 \pm 0.12$ ). Increased levels of serum creatinine in methanolic extract treated groups are controlled equal to normal group values are represented in table 1 and figure 1.

**Table 4: Effect of methanolic extract of *c.cyminum* on urinary excretion of calcium**

| Groups | Treatment                                               | Calcium (mg/dl)        |
|--------|---------------------------------------------------------|------------------------|
| I      | Normal group                                            | $10.02 \pm 0.24$       |
| II     | Inducer group                                           | $22.02 \pm 0.36$       |
| III    | Standard group                                          | $12.77 \pm 0.18^{***}$ |
| IV     | Methanolic extract of <i>c. cyminum</i> (200mg/kg) (CR) | $17.59 \pm 0.13^{***}$ |
| V      | Methanolic extract of <i>c. cyminum</i> (400mg/kg) (CR) | $16.12 \pm 0.12^{***}$ |
| VI     | Methanolic extract of <i>c. cyminum</i> (200mg/kg) (PR) | $15.11 \pm 0.13^{***}$ |
| VII    | Methanolic extract of <i>c. cyminum</i> (400mg/kg) (PR) | $13.58 \pm 0.17^{***}$ |



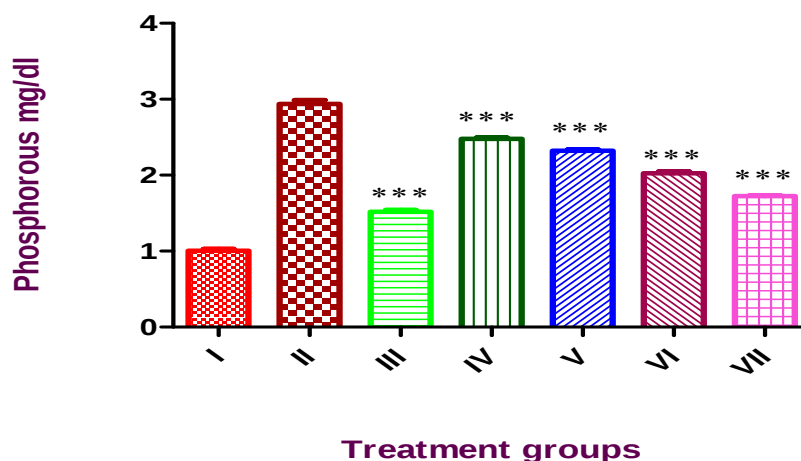
**Figure 16: Methanolic extract of *c. cyminum* on urinary excretion of calcium.**

Values are expressed as Mean  $\pm$  SEM (n=6).

P-value \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 significant. Comparisons were done on the induced group vs. drug treated group. Ns – Non significant.

**Table 5: Effect of methanolic extract of *c. cyminum* on urinary excretion of phosphorous**

| Groups | Treatment                                               | phosphorous (mg/dl) |
|--------|---------------------------------------------------------|---------------------|
| I      | Normal group                                            | 1.005 $\pm$ 0.02    |
| II     | Inducer group                                           | 2.938 $\pm$ 0.05    |
| III    | Standard group                                          | 1.520 $\pm$ 0.02*** |
| IV     | Methanolic extract of <i>c. cyminum</i> (200mg/kg) (CR) | 2.477 $\pm$ 0.01*** |
| V      | Methanolic extract of <i>c. cyminum</i> (400mg/kg) (CR) | 2.323 $\pm$ 0.01*** |
| VI     | Methanolic extract of <i>c. cyminum</i> (200mg/kg) (PR) | 2.025 $\pm$ 0.02*** |
| VII    | Methanolic extract of <i>c. cyminum</i> (400mg/kg) (PR) | 1.723 $\pm$ 0.01*** |



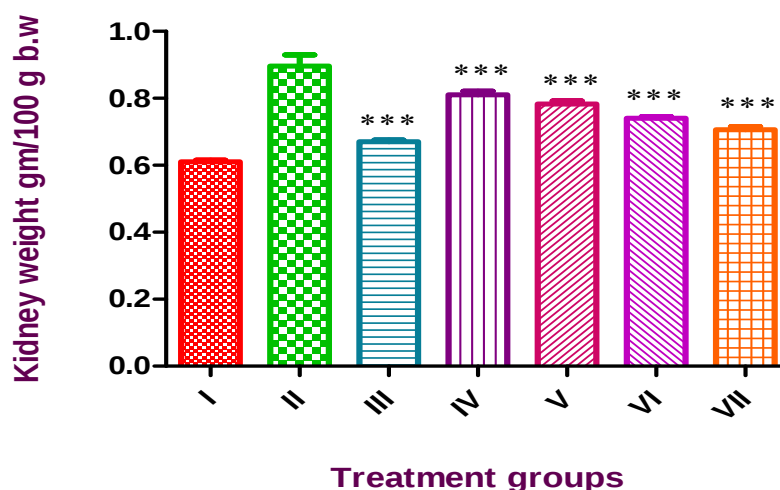
**Figure 17: Methanolic extract of *c. cyminum* on urinary excretion of phosphorous.**

Values are expressed as Mean  $\pm$  SEM (n=6).

P-value \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 significant. Comparisons were done on the induced group vs. drug treated group. Ns – Non significant.

Table 6: Effect of methanolic extract of *c.cyminum* on kidney weight

| Group | Treatment                                               | Kidney weight gm/100g. b.w. |
|-------|---------------------------------------------------------|-----------------------------|
| I     | Normal group                                            | 0.61±0.005                  |
| II    | Inducer group                                           | 0.89±0.032                  |
| III   | Standard group                                          | 0.67±0.005***               |
| IV    | Methanolic extract of <i>c. cyminum</i> (200mg/kg) (CR) | 0.81±0.011***               |
| V     | Methanolic extract of <i>c. cyminum</i> (400mg/kg) (CR) | 0.78±0.008***               |
| VI    | Methanolic extract of <i>c. cyminum</i> (200mg/kg) (PR) | 0.74±0.005***               |
| VII   | Methanolic extract of <i>c. cyminum</i> (400mg/kg) (PR) | 0.70±0.008***               |

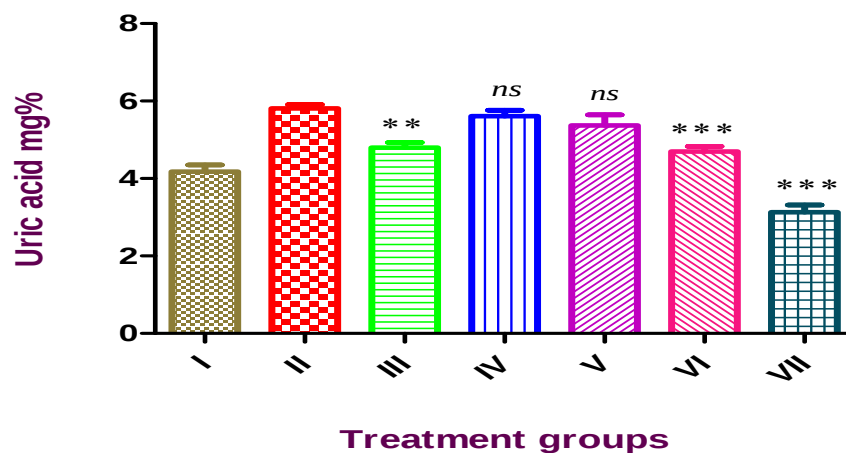
Figure 18: Methanolic extract of *c. cyminum* on kidney weight.

Values are expressed as Mean  $\pm$  SEM (n=6).

P-value \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 significant. Comparisons were done on the induced group vs. drug treated group. Ns – Non significant.

Table 7: Effect of methanolic extract of *c.cyminum* on uric acid

| Group | Treatment                                               | Uric acid (mg%)           |
|-------|---------------------------------------------------------|---------------------------|
| I     | Normal group                                            | 4.175±0.175               |
| II    | Inducer group                                           | 5.812±0.095               |
| III   | Standard group                                          | 4.797±0.131**             |
| IV    | Methanolic extract of <i>c. cyminum</i> (200mg/kg) (CR) | 5.613±0.148 <sup>ns</sup> |
| V     | Methanolic extract of <i>c. cyminum</i> (400mg/kg) (CR) | 5.367±0.278 <sup>ns</sup> |
| VI    | Methanolic extract of <i>c. cyminum</i> (200mg/kg) (PR) | 4.700±0.127***            |
| VII   | Methanolic extract of <i>c. cyminum</i> (400mg/kg) (PR) | 3.132±0.182***            |



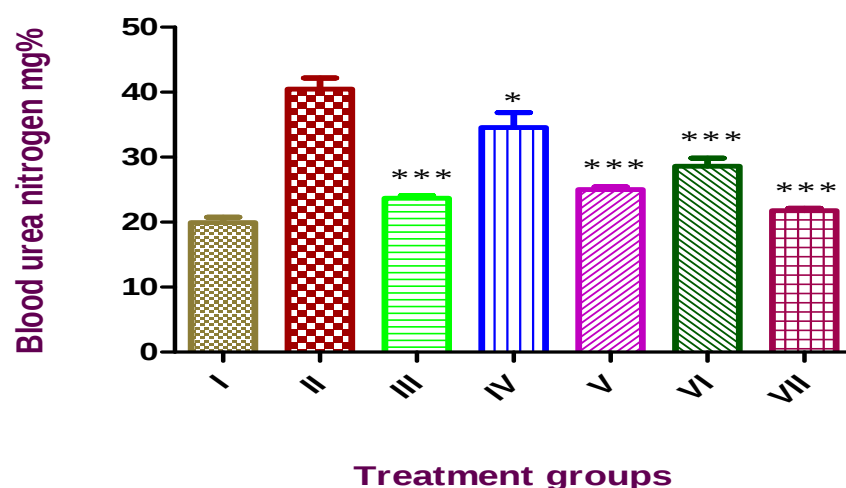
**Figure 19: Methanolic extract of *c. cyminum* on uric acid.**

Values are expressed as Mean  $\pm$  SEM (n=6).

P-value \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 significant. Comparisons were done on the induced group vs. drug treated group. Ns – Non significant.

**Table 8: Effect of methanolic extract of *c. cyminum* on Blood urea nitrogen**

| Group | Treatment                                               | Blood urea nitrogen (mg%) |
|-------|---------------------------------------------------------|---------------------------|
| I     | Normal group                                            | 19.95 $\pm$ 0.805         |
| II    | Inducer group                                           | 40.47 $\pm$ 1.702         |
| III   | Standard group                                          | 23.68 $\pm$ 0.419***      |
| IV    | Methanolic extract of <i>c. cyminum</i> (200mg/kg) (CR) | 34.55 $\pm$ 2.325*        |
| V     | Methanolic extract of <i>c. cyminum</i> (400mg/kg) (CR) | 25.01 $\pm$ 0.422***      |
| VI    | Methanolic extract of <i>c. cyminum</i> (200mg/kg) (PR) | 28.61 $\pm$ 1.248***      |
| VII   | Methanolic extract of <i>c. cyminum</i> (400mg/kg) (PR) | 21.76 $\pm$ 0.365***      |



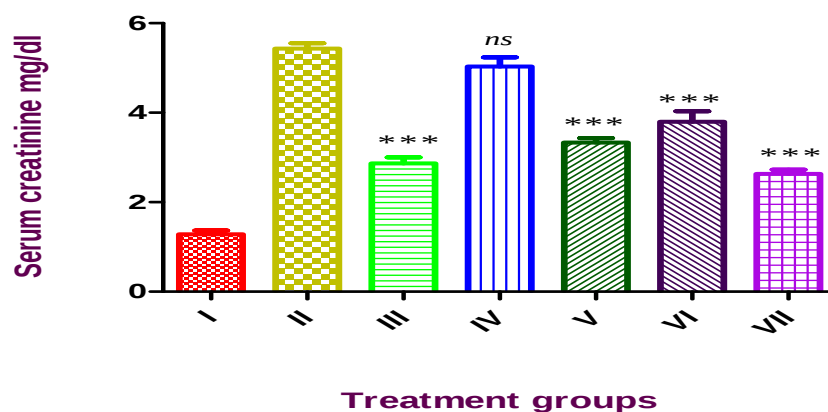
**Figure 20: Methanolic extract of *c. cyminum* on blood urea nitrogen.**

Values are expressed as Mean  $\pm$  SEM (n=6).

P-value \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 significant. Comparisons were done on the induced group vs. drug treated group. Ns – Non significant.

**Table 9: Effect of methanolic extract of *c.cyminum* on Serum creatinine**

| Group | Treatment                                               | Serum creatinine (mg%)    |
|-------|---------------------------------------------------------|---------------------------|
| I     | Normal group                                            | 1.283±0.083               |
| II    | Inducer group                                           | 5.433±0.120               |
| III   | Standard group                                          | 2.867±0.133***            |
| IV    | Methanolic extract of <i>c. cyminum</i> (200mg/kg) (CR) | 5.033±0.202 <sup>ns</sup> |
| V     | Methanolic extract of <i>c. cyminum</i> (400mg/kg) (CR) | 3.333±0.988***            |
| VI    | Methanolic extract of <i>c. cyminum</i> (200mg/kg) (PR) | 3.800±0.230***            |
| VII   | Methanolic extract of <i>c. cyminum</i> (400mg/kg) (PR) | 2.633±0.095***            |

**Figure 21: Methanolic extract of *c. cyminum* on serum creatinine.**

Values are expressed as Mean ± SEM (n=6).

P-value \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 significant. Comparisons were done on the induced group vs. drug treated group. Ns – Non significant.

## 7. DISCUSSION

Urinary calculi are the third prevalent disorder in the urinary system (Hadjzadeh et al., 2007). Most calculi in the urinary system arise from a common component of urine, e.g. calcium oxalate representing up to 80% of analyzed stones (Karadi et al., 2006). 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 (Joseph et al., 2005).

Urolithiasis refers to the solid nonmetallic minerals in the urinary tract. Among the several types of kidney stones, the most common are calcium oxalate. The formation of these stones involves several physicochemical events, beginning with crystal nucleation, aggregation, and ending with retention within the urinary tract (Khan et al., 1997).

In the presented study, male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans (Vermeulen, 1962).

Urinary supersaturation with respect to stone forming constituents is generally considered to be one of the causative factors in calculogenesis. Evidences in

previous studies indicate that in response to 28 days period of ethylene glycol (0.75%, v/v) administration. Stone formation in ethylene glycol fed animals is caused by hypercalciuria, which causes increased renal retention and excretion of calcium.

In the present study, calcium and phosphorous excretion are progressively increased in calculi induced animals. Since it is accepted that hypercalciuria is the significant risk factor for the pathogenesis of stone formation.

Increased urinary calcium is a factor favoring the nucleation and precipitation of calcium phosphate (apatite) from urine and subsequent crystal growth. The increased urinary excretion of calcium and phosphorous levels in urolithiatic rats similar to that of Christiana et al., 2002; Karadi et al., 2006; Bahuguna et al., 2010. Were showed urolithiatic rats increases the excretion of calcium and phosphorous.

In urolithiasis, the glomerular filtration rate (GFR) decreases due to the obstruction to the outflow of urine by stones in urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine and uric acid get accumulated in blood. In ethylene glycol induced rats there is an increased

levels of BUN, serum creatinine and uric acid were observed. However, the curative and preventive treatment with methanolic extract of *c. cyminum* reduces the levels of BUN, serum creatinine and uric acid, which are similar to that of Bahuguna et al., 2010.

## CONCLUSION

Methanolic extract of *c. cyminum* Linn, significantly reduced the elevated levels of calcium, phosphorous, blood urea nitrogen, uric acid & serum creatinine in curative & preventive treatment groups. The histopathological findings also show sign of improvement after treatment with the methanolic extract of *c. cyminum*. All these observation provided that the basis for the conclusion that *c. cyminum* Linn seed extract inhibited the stone formation induced by ethylene glycol treatment.

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