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EVALUATION OF ANTI-DIURETIC AND ANTI NEPHROLITHIATIC ACTIVITIES OF ETHANOLIC SEEDS EXTRACT OF *CICER ARIETINUM* IN EXPERIMENTAL RATS

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ABSTRACT

This study was aimed to evaluate the Diuretic activity and anti-Nephrolithiasis activities of Cicer arietinum's Ethanolic seed extract on albino rats. The seeds of Cicer arietinum were collected from the local market. It belongs to the family Fabaceae. The activity was studied by using ethylene glycol induced nephrolithiasis model. The standard drug used in the present study was Cystone. Two test doses of Cicer arietinum's Ethanolic seed extract were used. The total duration of evaluation was 28 days. Urine volume, Urine analysis, serum analysis were used to assess the efficacy of test drug. The results concluded that the decrease of urinary stones in the kidney and showed good diuretic property.

Key words: Diuretic activity, Anti- Nephrolithiasis, Cicer arietinum, Urine volume, Urine analysis, Serum analysis.

INTRODUCTION

Diuretics are the drugs which increase the renal excretion of water and sodium salts. Major purposes of diuretic therapy are to decrease fluid volume of the body, and to adjust the water and electrolyte balance. Anti-nephrolithiatics are the drugs which will reduce the urinary stones significantly. From the ancient days mankind facing a lot of problem with Lithiasis in Kidneys. Nowadays many drugs are available to treat urinary failure. Medical management of lithiasis, today, includes lithotripsy and surgical procedures¹. Herbal medicines significantly acting on the

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kidneys and shows good diuretic property along with anti-nephrolithiasis activity. In the present study we were evaluated the activity of Ethanolic seeds extract of *Cicer arietinum* against ethylene glycol induced albino Rats. Now the current study is aimed to evaluate the Diuretic and Anti-Nephrolithiasis effect of Ethanolic seeds extract of *Cicer arietinum*.

MATERIALS AND METHODS

Drugs and Chemicals

Cystone was purchased at Himalaya Store, Begumpet, and Hyderabad. Ethylene glycol was

purchased from Prime Laboratories, Himayatnagar, Hyderabad-63. Distilled water- central drug house (p) ltd, New Delhi.

Plant material and preparation of extract²

The seeds of *Cicer arietinum* (organic variety) were collected from the market, Miyapur, Hyderabad. The seeds of *Cicer arietinum* were pulverized into fine powder along with the husk and used for extraction. The powdered dried seeds were loaded into the Soxhlet extractor and subjected to extraction with 99% ethanol for 18 hrs. At 72^o C. After extraction the solvent was distilled off and the extract was concentrated to dryness at room temperature.

Experimental animals

Albino rats of either sex of weighed between 150-250 Gms were taken from the Animal House of Albino research training Institute, Bachupally, and HYD-90. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^oC and relative humidity of 30-70 %. A 12:12 light: day cycle was followed.

EXPERIMENTAL

Phytochemical analysis

The Ethanolic seeds extract of *Cicer arietinum* were subjected to preliminary phytochemical screening and phytochemical reported in the seeds of *Cicer arietinum* were Flavonoids, phenols, Carbohydrates, Saponins.³

Acute toxicity studies⁴

Acute oral toxicity studies of the extracts were carried out as per the OECD guidelines, draft guidelines 423 adopted and received from Committee for the Purpose of Supervision and Control of Experiments on Animals (CPCSEA), Ministry of social justice and empowerment, Government of India. The results were mentioned in table no.1.

Experimental design

Wistar albino rats weighed between 150-250gms were used in the present study. All the animals were randomly divided into the six groups

each group consists of 6 animals and they have received the treatment as follows

Group I : Normal vehicle Control received Distilled water p.o.

Group II: Animals received Ethylene glycol 0.5ml/day in drinking water⁵

Group III: Animals received Ethylene glycol and standard drug Cystone (500mg/kg p.o)

Group IV: Animals received Ethanolic extract of *Cicer arietinum* (250mg/kg p.o.)

Group V: Animals received Ethanolic extract of *Cicer arietinum* (500mg/kg p.o).

- Animals received Ethylene glycol 0.5ml/day in drinking water for 28 days.
- Standard and two doses of test drug were given from 15th day till 28th days.⁶

Assessment of Diuretic activity and Anti-Nephrolithiasis activity.

Urine volume

Each group of animals was placed in separate metabolic cages for 24 hrs. After 24hrs the total urinary volume was measured using the measuring cylinder which was kept below the every cage and measured in ml.

Collection and analysis of urine:

Each group of animals was placed in separate metabolic cages for 24 hrs. and urine samples were collected on 28th day. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4^oC. Urine samples were analyzed for Sodium, Potassium, calcium, creatinine, and urea.

Statistical analysis

The data were presented as Mean± SEM and statistical analysis was done by using Graph pad prism software and results were analysed by one way ANOVA followed by Dunnett's multiple comparison test. The obtained results were compared with toxic control group and P<0.05, P<0.01 is considered as significant.

RESULTS

In the present study the chemical which is used developed the urinary stone. Ethylene glycol (0.5ml) produces hyperoxyluria. From table no.1 there is no mortality was observed in acute toxicity studies. After the completion of 28 days of

evaluation the Diuretic activity of two test doses of the drug was observed. From the table no.2 the Urine volume was measured and compared with the control group significant Diuretic property was exerted by the test drug. The urine volumes were increased significantly by the standard drug Cystone. From table no.3 the Urine biochemical parameters were estimated and significant output was observed from the two doses of the test drug and standard drug.

DISCUSSION

The chemical which is used in the present study to produce lithiasis in kidneys effectively showed its activity. Kidney being the target for ethylene glycol toxicity it shows some changes in its metabolism while the 4 weeks of continuous treatment. There is a formation of micro crystals was observed⁷. After the treatment of 0.5ml of ethylene glycol renal calculi composed of calcium oxalate crystals was observed⁸. Due to the formation of stones in the kidneys the urine retention was excess⁹. In the present study group-ii is used as control group. So

we could clearly observe the changes when compared the control group with the standard and test groups. Due to the formation of stones the urine retention increases. So, glomerular filtration decreases gradually. There by there was an accumulation of nitrogenous waste substances in the kidney^{10, 11}. The standard drug used in the present study called Cystone (500mg/Kg p.o) has showed significant changes in the Urine volume, Urinary biochemical parameters and Salts present in the urine. It exerts good diuretic property. The test doses after treatment of 28 days produced significant output. The diuretic property was compared with the standard drug Cystone and observed elevated urine volume.

CONCLUSION

From the above obtained results the Ethanolic seeds extract of *Cicer arietinum* had potent Diuretic and Anti-Nephrolithiasis activity. So the present test drug is safe and having good therapeutic efficacy.

Table 1. Effect of ethanolic extract of *Cicer arietinum* in acute toxicity for 48 hrs.

DOSE	NO OF RATS/NO.OF MORATLITY			
	6hr	12hr	24hr	48hr
250mg/kg	6/0	6/0	6/0	6/0
500mg/kg	6/0	6/0	6/0	6/0
750mg/kg	6/0	6/0	6/0	6/0
1000mg/kg	6/0	6/0	6/0	6/0
2000mg/kg	6/0	6/0	6/0	6/0

Table 2: Effect of oral administration Ethanolic seeds extract of *Cicer arietinum* on urinary volume excretion

Group	Urine volume(ml/100g/24hr)
Normal	4.5±0.12
Control (Ethylene glycol 0.5ml/day in drinking water)	1.7±0.04
Standard (Ethylene glycol and standard drug Cystone (500mg/kg p.o))	7.33 ± 0.31***
Test-1 (250mg/kg)+ Ethylene glycol	5.0±0.04**
Test-2 (500mg/kg)+ Ethylene glycol	6.4±0.02***

Values are expressed as Mean ± SEM, (n=6), *P<0.05, ** P<0.01, *** P<0.001

Table No. 3: Effect of Ethanolic seeds extract of *Cicer arietinum* urine biochemical

Treatment	Na+ m.mol/L	K+ m.mol/L	Cl ⁻ m.mol/L	Calcium (mg/dl)	Urea (mg/dl)	Creatinine (mg/day)
Normal	95±0.02	92±1.4	55±2.1	1.12±0.5	24.12±0.1	2.25±0.03
Control (Ethylene glycol 0.5ml/day in drinking water)	71±0.04	65±1.8	74±1.4	5.1±0.05	48±0.03	5.12±0.1
Standard (Ethylene glycol and standard drug Cystone (500mg/kg p.o))	125±0.1***	85±0.9***	57±0.75***	1.4±0.03***	32.2±0.04***	3.5±0.14***
Test-1 (250mg/kg)+ Ethylene glycol	112±1.2**	71±1.23	61±1.11**	2.1±0.01**	38.15±0.1**	4.1±0.2**
Test-2 (500mg/kg)+ Ethylene glycol	121±1.4***	77±1.44**	58±1.31***	1.8±0.06***	35.2±0.2***	3.8±0.3***

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