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EVALUATION OF ANTINEPHROLITHIATIC ACTIVITY OF ETHANOLIC EXTRACT OF *Rosa indica Linn* ON ETHYLENE GLYCOL INDUCED MALE WISTAR ALBINO RATS

P Amudha[1*], Nagalakshmi Swetha Y[2] and Ghanapiyari Sharma[2]

[1]Asst. Professor, [2]Department of Pharmacology C. L. Baid Metha College of Pharmacy, Thoraipakkam, Chennai, Tamil Nadu, India.

ABSTRACT

Kidney stone disease has afflicted humankind since antiquity and can persist, with serious medical consequences, throughout a patient's lifetime. The study was designed to evaluate prophylactic effect of 28 days treatment of ethanolic extract of *Rosa indica EERI* (200 mg/kg and 400 mg/kg, p.o.) against ethylene glycol induced nephrolithiasis in rats and thiazide (0.9mg/kg p.o) was used as a standard. After completion of 28 days treatment body weight, food intake, pH, urinary calcium, urinary magnesium and biochemical parameters like Urea, creatinine, SGOT, SGPT, ALP and LDH from blood serum were measured. Lithogenic treatment with ethylene glycol caused damage of kidney when it is metabolised by the body, it produces three toxic metabolites like glycoaldehyde, glycolate and glyoxylate, which formed calcium oxalate crystal deposition in kidney and increased in hyperoxaluria, hypercalciuria and hypomagnesaemia contributing to renal stone formation which was prevented by simultaneous administration of EERI thereby reduces the growth and development of kidney stones forming promoters like calcium, phosphate, creatinine, urea, and enhanced stone forming inhibitors like magnesium, renal and hepatic impairment with significant antioxidant activity in a dose dependent manner. Antiurolithiatic effect of EERI was also confirmed by histopathological changes in kidney tissue. The results of the present study indicated antiurolithiatic activity of EERI which was comparable to thiazide.

Key words: Antiurolithiatic activity, Ethanolic extracts of *Rosa indica linn* (EERI), Ethylene glycol, calcium oxalate crystal, Nephrolithiasis.

INTRODUCTION

The urinary system or renal system is the organ system that produces, store and eliminates urine. It includes kidneys, ureters, bladder and urethra (Dugdale and David, 2011). Nephrolithiasis is a process of forming a kidney stone, a stone in the kidney. These stones are called as renal calculi which is very common and painful kidney disorder. They are formed when urine becomes concentrated, allowing minerals to crystallize and stick together which forms microscopic crystals in the loop of henle, distal tubules, or the collecting duct. This is usually in response to elevated levels of urinary solutes, such as calcium, uric acid, oxalate, and sodium, as well as decreased levels of stone inhibitor, such as citrate and magnesium, these can lead to urine super saturation and subsequent stone formation (Worcester EM, 1996). If left untreated it can cause complication like permanent kidney damage, urethritis, cystitis, pyelonephritis, urinary tract blockage, infection, urinary fistula. The prevalence of urinary calculi is estimated to be range between 4%-20% in the general population (Trinchieri A, 1996). Treatments are different according to the type of stones i.e. for calcium oxalate stones diuretics, citrate salts, phosphates and cholestryamine are used, for uric acid stones Potassium citrate, sodium bicarbonate and allopurinol are used, for struvite stones Antibiotics to eliminate infection and for cystine stones alkalizing agents such as bicarbonate, d-penicillamine, tiopronin or captopril are

Corresponding Author

P Amudha
Email: amudha.cology@gmail.com

used (Wen CC and Nakada SY, 2007). The drugs currently used include thiazides, potassium citrate, and allopurinol (Dalbeth *et al*., 2007). Recently there has been increase interest in use of traditional medicine which includes treatment for kidney stones. Compare to synthetic drug, drugs derived from plant are frequently considered to be less toxic with few side effects. Traditionally the rose flower, *Rosa indica* Linn is an important shrub, used in siddha system of medicine since ages. They have antimicrobial activities, astringent, cardiac trouble, as tonic, against asthma, cough, bronchitis, fever, wounds, hyperhydrosis, sprains, ulcers, general debility and also used for the removal of gal bladder and kidney stone (Heywoood and Vernon H, 1993).

MATERIALS AND METHODS

Plant materials

The flowers of *Rosa indica Linn* (Rosaceae) was collected and authenticated by Dr.D.Aravind, Assistant Professor, National Siddha Hospital, Chennai-47. Dried drug sample was powdered coarsely with an electronic blender and about 600g of this powder was macerated with 95% ethyl alcohol for 72 hrs. EERI was then dried in vacuum and store at 0-4^{0}C and resuspended in water before use.

Preliminary Phytochemical Analysis

EERI was subjected to preliminary phytochemical screening for the presence or absence of phytoconstituents like alkaloids, carbohydrates, steroids, proteins, tannins, phenols, flavonoids, flavanones, gum and mucilage, glycosides, saponins, terpenes and sterols (indianfood.com/rose.htm).

Experimental animal studies

Adult female wistar rats of weighing 200-250 gm were used for the study. They were housed 6 per cage under standard laboratory condition at a room temperature at 22$\pm$2^{0}C with 12hrs light/dark cycle and were provided with standard pellet chow and water ad libitum.

Acute oral toxicity studies

The toxicity studies were done using OECD 423 guidelines. The method used defined dose (2000mg/kg body weight), results allow a substance to be ranked and classified according to the globally harmonized system (GHS) for classification of chemical which cause acute toxicity. The starting dose level of EERI was 2000mg/kg body weight p.o. Dose volume administered was 1ml/100gm body weight to fasted female rats with 1%w/v CMC. Food was withheld for a further 3-4hrs after administration of drugs and observed for the onset and signs of toxicity. Body weight of rats before and after determination were noted, any changes in skin, fur, eyes, mucous membrane, respiratory, circulatory, central nervous system, motor activity, behaviour pattern and also sign of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma were observed.

Experimental procedure

Thirty healthy adult male wistar rats were divided into 5 groups each contains 6 animals. Stones were induced in rats by giving ethylene glycol 0.75 ml in 100 ml of drinking water per day orally upto 28 days (Patel PK *et al*., 2012). At the end of the study on 29th day, urine was collected for the estimation of calcium and magnesium and the animals were sacrificed under light ether anaesthesia and blood was collected for the estimation of biochemical parameters. Right kidney was examined for the presence of calcium oxalate crystals and stone formation by histological techniques

Group I: Control animals.

Group II: Treated with Ethylene glycol 0.75%v/v in drinking water

Group III: Treated with Ethylene glycol 0.75%v/v and Thiazide 0.9mg/kg p.o

Group IV: Treated with Ethylene glycol 0.75%v/v and EERI (200mg/kg p.o)

Group V: Treated with Ethylene glycol 0.75%v/v and EERI (400mg/kg p.o)

Biochemical studies

The animals were sacrificed on 29th day by decapitation and blood was collected by bleeding of retro orbital plexus using micro capillary technique from all the group of overnight fasted rats. The serum was separated for the estimation of SGOT (Ecobichon DJ, 1997), SGPT (Ananta Teepa *et al*., 2010), Creatinine (Toxicology & Neurosciences Center, Shreveport., 2009), urea (Bablok W et.al., 1988), alkaline phosphatase (Glick MR *et al*., 1986), lactate dehydrogenase (Browers, 1980). 24 hours urine was collected for estimation of calcium (Chaney AL and Marbach EP., 1962), magnesium (Bablok W *et al*., 1988) were estimated by using standard procedure.

RESULTS

EERI reveals the presence of Alkaloids, Flavonoids, glycoside, Tannins, Reducing sugars, Steroids, Terpenoids, Saponins and showed absence of carbohydrates, proteins, phenols, flavanones, gum, mucilage, terpenes and sterols.

Acute oral toxicity was done according to the OECD 423 guidelines. There was no morbidity and mortality, no considerable change in body weight after treatment and no signs of toxicity was observed in female rats. Results are shown in Table 1.

There was significant ($p<0.001$) decrease in body weight, food intake and pH in group II when compared to group I. There was significant ($p<0.001$)

decrease in body weight and food intake in group III, group IV and group V when compare to group I. There was significant ($p<0.05$) decrease in pH in group III and ($p<0.001$) group IV whereas showed significant ($p<0.05$) increase in pH in group V when compared to group I. There was significant ($p<0.001$) increase in body weight and food intake in group III, group IV and group V when compare to group II. There was significant ($p<0.001$) increase in pH in group III, group V and ($p<0.05$) group IV when compared to group II. Results were shown in Table 2 and Figure 1

There was significant ($p<0.001$) increase in serum Urea, Creatinine, SGOT, SGPT, ALP, LDH and Calcium in group II, group III, group IV and group V when compared to group I. There was significant ($p<0.001$) in magnesium in group II and decrease ($p<0.01$) in magnesium in group III, group IV and group V when compared to group I.There was significant ($p<0.001$) decrease in urea, creatinine, SGOT, SGPT, ALP, LDH and calcium in group III and group V and decrease ($p<0.01$) in group IV when compared to group II. There was significant increase ($p<0.01$) in magnesium in group III, group V and ($p<0.05$) group IV when compared to group II. Results were shown on Tables 3 & 4 and Figure 2,3&4.

Figure 1. Effect of EERI on LDH, Body weight and Food intake

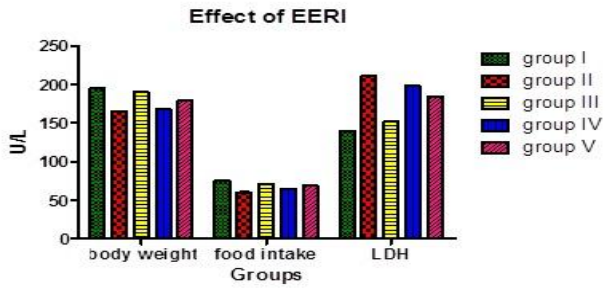


Figure 2. Effect of EERI on Urea, Calcium and pH

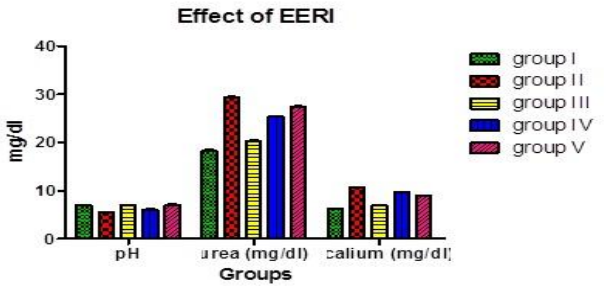


Figure 3. Effect of EERI on creatinine and magnesium

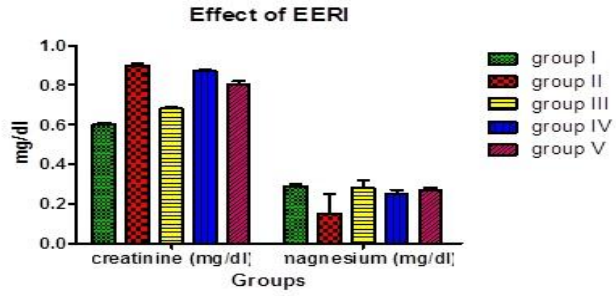
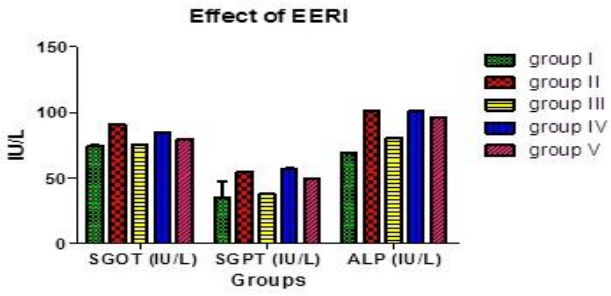


Figure 4. Effect of EERI on SGOT, SGPT and ALP



The values were expressed as mean ±EM of 6 animals. Comparisons were made between as a- Group I vs group II, III, IV, V and b- Group II vs Group III, IV, V. Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test. P*<0.05 P**<0.01 P***<0.001 ns-non significance.

Table 1. Acute oral toxicity effect of EERI

SL. NO.	Treatment Group	Dose	Weight of Animal In gms		Signs Of toxicity	Onset Of toxicity	Duration
			Before test	After test			
1.	EERI	2g/kg	200	200	No signs of toxicity	Nil	14 days
2.	EERI	2g/kg	200	200	No signs of toxicity	Nil	14 days
3.	EERI	2g/kg	250	250	No signs of toxicity	Nil	14 days

Table 2. Effect of EERI on body weight, food intake and LDH

Groups	Body weight(gms)	Food intake(gms)	LDH (U/L)
I	195±0.19	75.4±0.36	140.25±0.02
II	165±0.17a***	60.48±0.24a***	212.04±0.06a***b***
III	190±0.12 a***b***	70±0.3a***b***	152.25±0.01a***b***
IV	168±0.02 a***b***	64.7±0.06 a***b***	198.75±0.06 a***b***
V	179±0.07 a***b***	68.8±0.25 a***b***	185.65±0.01 a***b***

Table 3. Effect of EERI on pH, Urea, creatinine and calcium

Groups	pH	Urea (mg/dl)	Creatinine (mg/dl)	Calcium (mg/dl)
I	7.0±0.03	18.25±0.21	0.600.01	6.330.01
II	5.5±0.13a***	29.3±0.2a***	0.90.016a***	10.70.01a***
III	7.0±0.01a*b***	20.2±0.1a***b***	0.60.013a***b***	7.00.05a***b***
IV	6.0±0.15 a***b*	25.3±0.1a***b***	0.80.01a***b*	9.70.03a***b*
V	7.0±0.21 a*b***	27.3±0.2a***b***	0.80.02a***b***	8.90.01a***b**

Table 4. Effect of EERI on magnesium, SGOT, SGPT and ALP

Groups	Magnesium (mg/dl)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
I	0.29±0.01	74.7±0.12	35.06±0.1	68.8±0.01
II	0.15±0.3a***	99.9±0.02a***	54.7±0.01a***	1012.7±0.04a***
III	0.28±0.04a**b***	75.4±0.07a***b***	37.8±0.03a***b***	80.7±0.2a***b***
IV	0.25±0.02a*b*	84.7±0.1a***b***	56.7±0.7a***b***	100.8±0.02a***b**
V	0.27±0.01a**b**	79.7±0.01a***b***	49.4±0.1a***b***	95.8±0.01a***b***

DISCUSSION AND CONCLUSION

Stones may occur in any part of the urinary system like kidney, ureter, bladder and its one of the most painful disease. The prevalence of urinary calculi is estimated to be range between 4%-20% in the general population[5]. Urinary lithiasis is due to imbalance between inhibitors and promoters in the kidney. Urinary supersaturation is the driving force behind crystal formation in the kidney which is called as nucleation[3]. Once a crystal nucleus has achieved a critical size, crystals in solution stick together to form larger particles called as aggregation which served as important step in stone formation (Pearle M and Lotan Y., 2002). Diuretics and potassium citrate are the major class of drugs used in nephrolithiasis. In spite of tremendous advance in the field of medicine, there is truly no satisfactory drug for the treatment of nephrolithiasis. Recently there is increasing evidence that many healthy natural food and medicinal herbal and supplements have the potential to become valuable complementary therapy in the treatment of various renal disorder and protection against nephrotoxicity. The plant *Rosa indica* linn is traditionally used for different disorder such as antimicrobial activity, skin irritation, gal stones, rheumatism & gout. An attempt was made to study nephrolithiatic activity in ethylene glycol induced method. Ethylene glycol is metabolised by the body, produced three toxic metabolite like glycoaldehyde, glycolate and glyoxylate, which causes tissue destruction by forming calcium oxalate crystal deposition in kidney results in renal failure, hyperoxaluria and stone formation which was treated by the administration of EERI. The preliminary phytochemical analysis of EERI showed the presence of alkaloids, cardiac glycoside, reducing sugars, steroids & terpenoids, saponins, tannins, flavanoids which are used against nephrolithiasis. Acute toxicity studies revealed that the EERI did not produces any mortality and morbidity or signs of toxicity at the dose of 2000mg/kg p.o in experimental animals. Treatment with EERI showed increased in food intake, body weight and urinary pH. Biochemical studies revealed that EERI showed decreased in SGOT, SGPT, ALP, serum urea, creatinine and LDH. Urinary analysis showed the decrease in calcium level and increase in magnesium level in both doses further confirms the antinephrolithiasis activity. This activity may be due to the presence of flavanoids and tannins. The histopathological studies suggested that no microcrystalline deposition and kidney damage in the *Rosa indica* Linn extract treated group. The present study indicates the administration of EERI in ethylene glycol induced nephrolithiasis reduces the growth and development of kidney stones, renal and hepatic impairment. Accordingly, it can be concluded that the supplementation of *Rosa indica* Linn has beneficial effect on nephrolithiasis.

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