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Research Article

Evaluation of Diuretic and Antinephrolithiatic Activity of *Cucurbita pepo* Seed in Experimental Rats

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Abstract

This study was aimed to evaluate the effectiveness of the *cucurbita pepo* seed on albino rats as a preventive agent against the development of kidney stones. The seeds of *cucurbita pepo* were collected from the local market of Lalitpur, district (UP). Activity of *cucurbita pepo* seed was studied using the ethylene glycol-induced nephrolithiasis model. Standard drug used was cystone. Several parameters were used including urinary volume, urine analysis and serum analysis to assess the activity. The results indicated that the administration of *cucurbita pepo* to rats with ethylene glycol-induced lithiasis significantly reduced and prevented the growth of urinary stones ($P < 0.01$). Also, the treatment of lithiasis-induced rats by *cucurbita pepo* seed restored all the elevated biochemical parameters (calcium, creatinine, uric acid and urea), increased the urine volume significantly ($P < 0.01$) when compared to the model control drug. This study supports the usage of *cucurbita pepo* seed in nephrolithiasis and the utility could further be confirmed in other animal models.

Keywords: Antinephrolithiatic Activity, Ethylene glycol, *Cucurbita pepo,* Renal calculi.

INTRODUCTION

Urinary stone disease has affected humankind since antiquity and can persist, with serious medical consequences, throughout the patient's lifetime. In addition, the incidence of kidney stones has been increased in western societies in the last five decades, in association with economic development[1-3]. An important reason for acute and chronic renal failure, lithiasis (stone formation), includes both nephrolithiasis (stone formation in kidney) and urolithiasis (stone formation in ureter or bladder or bladder or both). Among the various kinds of stones identified, calcium stones occur mainly in men, while phosphate stones happen more in women. The pathogenesis of lithiasis seems to be multifactorial and complicated. Medical management of lithiasis, today, includes lithiotripsy and surgical procedures[4] and local calculus disruption using high-power laser are widely used to remove the calculi[5]. Many remedies have been employed since ages to treat renal stones and most of them were from plants and proved to be useful[6]. The present day medical management of nephrolithiasis is either costly or not without side effects[7].The current study was aimed to evaluate the effectiveness of the *cucurbita pepo* seed on albino rats as a preventive agent against the development of kidney stones.

MATERIAL AND METHODS

Experimental animals

Adult male albino wister rats were used for this study with the weight range of 100-150gm.They were maintained under 12h dark/light cycle in well ventilated polypropylene metabolic cages (3-4/cage) at 25±2°C. They were fed with standard pellet diet and had free access to water. The animals were maintained in the above said conditions for a week before the experiment. This study has approval from Institutional Animal Ethics Committee (IAEC).

Plant material

The seeds of *cucurbita pepo* was collected from the local market of Lalitpur, district (UP) and authentified by Head of Department of botany, Dr. Zia ul Hasan, Professor of Saifia Science College, Bhopal with voucher Specimen No.347/Bot/Safia/2012.

Preparation of extract

The collected seeds of *cucurbita pepo* were washed well with water and dried under shade up to 15 to 20 days and then dried seeds were powdered with the help of grinder and mixer. Powdered material was then sieved by using 24 no. sieves. 50 grams of sieved powder was packed inside filter paper in the soxhlet tube and introduced in the extraction unit of Soxhlet extractor and extraction was done with ethanol and water for 25 cycles. The extracts were filtered and

concentrated with rotary evaporator. The concentrated product was then dried on a water bath.

Acute toxicity studies

Acute toxicity studies were carried out as per OECD guidelines (No: 423) using female Wister mice by employing the Up and Down method prior to evaluating each of the extracts for antinephrolithiatic activity. From acute toxicity test we concluded that (maximum dose-1/10th of maximum tolerable dose) i.e.(1000mg/kg) were found safe for the experimental animals.

Experimental design

Ethylene glycol-induced hyperoxaluria method was used to assess the antinephrolithiatic activity in albino Wister rats. Animals were divided in to five groups. Each groups containing six animals. Group I served as normal control. Group II Negative control received ethylene glycol 0.5ml/day in drinking water for induction of renal calculi for 28 days. Group III received ethylene glycol and standard drug cystone (750mg/kg p.o), from 15th day till 28th days. Group IV ethylene glycol and aqueous extract (100mg/kg p.o) from 15th to 28th days. Group V ethylene glycol and alcoholic extract (100mg/kg p.o) from 15th to 28th days[8].

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 28 th day. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. Urine samples were analyzed for calcium, creatinine, urea and uric acid.

Serum analysis

At the end of the experiment, blood samples were collected from the retro-orbital plexus under anesthetic conditions and analyzed for calcium, creatinine, urea and uric acid.

Urine volume

Animals were placed in separate metabolic cages for 24h and total urinary volume was measured using the measuring cylinder and reported in ml.

Statistical analysis

The results were expressed as the mean ± SEM and analyzed using one-way ANOVA followed by Dunnett's multiple comparison tests. Data were computed for statistical analysis using Graph Pad Prism Software and $P < 0.05$ was considered to be statistically significant.

RESULTS

In this study, the administration of 0.05% (v/v) ethylene glycol aqueous solution to male Wistar albino rats produced hyperoxaluria. Calcium excretion were grossly increased in the calculi-induced animals However, supplementation with *cucurbita pepo* significantly lowered the elevated level of calcium in urine when compared to the model control group [Table 1]. The level of serum calcium, creatinine, urea and uric acid were found to increase in the calculi-induced animal [Table 2]. In the case of *cucurbita pepo* treated groups, the treatment significantly (P < 0.001) lowered the elevated level of calcium, creatinine, urea and uric acid. Urine volumes were increased by *cucurbita pepo* and the standard drug cystone compared to the model control group.

Table: 1 Effect of *cucurbita pepo* extract on urine biochemical parameter in ethylene glycol induced antinephrolithiatic in rat

Sr no.	Group	Calcium(mg/dl)	Creatinine (mg/day)	Urea(mg/dl)	Uric acid (mg/dl)
1	Normal control	1.29±0.276	2.93±0.051	25.10±0.176	4.23±0.027
2	Negative control	4.83±0.766 a***	4.92±0.026 a***	45.75±0.225 a***	7.45±0.031 a***
3	Standard	1.57±0.261 a***b**	3.71±0.027 a**, b**	32.55±0.177 a**,b***	5.26±0.034 a***,b**
4	Test-1(aqueous)	1.74±0.461 a**,b**,c*	4.02±0.03 a** ,b**,c**	35.44±0.230 a**,b**,c*	5.52±0.027 a**,b**
5	Test-2(Alcoholic)	1.25±0.362 a***,b***,c**	3.81±0.028 a***, b***,c***	33.29±0.243 a***,b***,c**	5.37±0.032a***,b**,c**

Table: 2 Effect of *cucurbita pepo* extract on serum biochemical parameter in ethylene glycol induced antinephrolithiatic in rat

S.no.	Group	Calcium (mg/dl)	Creatinine (mg/dl)	Urea(mg/dl)	Uric acid (mg/dl)
1	Normal control	17.1±0.220	1.02±0.038	0.098±0.0012	0.028±0.007
2	Negative control	35.98±0.187 a***	2.45±0.135 a***	0.153±0.0012 a***	0.041±0.0087 a***
3	Standard	25.48±0.296 a***,b**	1.14±0.02 a**,b**	0.097±0.07 a**, b**	0.034±0.0012 a**,b*
4	Test-1(aqueous)	14.88±0.175 a***,b**,c**	1.29±0.038 a**,b***,c*	0.13±0.0099 a**,b**	0.033±0.0011 a**,b**
5	Test-2(Alcoholic)	11.038±0.224 a***b***,c***,d**	1.22±0.0381 a***,b**	0.12±0.0014 a***,b**,c**	0.030±0.006 a***,b**,c*

DISCUSSION

Different chemicals used to induce nephrolithiasis in experimental animals include ethylene glycol (EG), glycolic acid and ammonium oxalate[9]. Kidney, being the principle target for EG toxicity and its administration to the experimental animals for more than 4 weeks, resulted in substantial excretion of oxalate and deposition of microcrystal in kidneys[10].Therefore, in this study EG was preferred to induce nephrolithiasis. The chronic administration of 0.5% (v/v) ethylene glycol aqueous solution to albino rats produced hyperoxaluria. Calcium, was grossly increased in the calculi-induced animals However, supplementation with *cucurbita pepo* seed significantly lowered the elevated level of calcium in urine when compared to the standard group. The level of serum uric acid, urea and creatinine were found to increase in the calculi-induced animal. In the case of *cucurbita pepo* seed treated groups, the treatment significantly ($P < 0.001$) lowered the elevated level of creatinine, uric acid and urea. Urine volumes were increased by *cucurbita pepo* seed and the standard drug cystone compared to the model control group. This reinforces the *cucurbita pepo* seed diuretic property[11, 12]. Such an effect may be advantageous in the nephrolithiasis condition, as an increased urine output is recommended to reduce the possibility of stone formation. Urinary supersaturation with respect to stone forming constituents is generally considered to be one of the causative factors in calculogenesis. Evidence in previous studies indicated that after 14 days period of ethylene glycol (0.5%, v/v) administration, renal calculi were formed in the young male albino rat composed mainly of calcium oxalate[13]. Stone formation in ethylene glycol-fed rats is caused by hyperoxaluria, which cause increased renal retention[14]. In this study, calcium excretion was increased in calculi-induced animals (Group II). Treatment with the *cucurbita pepo* seed restored the calcium level, thus reducing the risk of stone formation[15]. In urolithiasis, the glomerular filtration rate (GFR) decreases due to the obstruction to the outflow of urine by stones in the urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine and uric acid get accumulated in blood. Also and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented with a calculi producing diet. In calculi-induced rats (Group II), marked renal damage was seen by the elevated serum and urine levels of creatinine and uric acid, and urea (Table1). However, aqueous and alcohol extracts of *cucurbita pepo* seed lower the levels of calcium and creatinine and uric acid and urea. The *cucurbita pepo* seed have positive effect in nephrolithiasis activity[16].

In this experimental study, the ethylene glycol induced nephrolithiasis, the *cucurbita pepo* seed, treated Group IV & V the treatment significantly ($P<0.001$) lowered the elevated level of group II. The results of the present study revealed that the alcohol extract of *cucurbita pepo* seed present a potent diuretic activity[17].

CONCLUSION

In conclusion, the results indicated that the administration of *cucurbita pepo* to rats with ethylene glycol-induced lithiasis reduced the growth of urinary stone. The dose requirement of *cucurbita pepo* seed to produce the activity is less than the standard drug used.

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