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Research Article

EVALUATION OF ANTI-UROLITHIATIC ACTIVITY OF CHLOROFORM AND METHANOLIC EXTRACT OF CUCUMIS MELO SEEDS AND FRUIT PEEL ON RATS

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ABSTRACT

Objective: Cucumis Melo is a traditional fruit belongs to family Cucurbitaceae. The fruit peel and seeds Cucumis melo were investigated for anti-urolithiatic activity.
Method: 0.75%v/v of Ethylene glycol was administered in drinking water for inducing renal calculi and increased renal excretion of urinary calcium and oxalate for 28 days. Cucumis melo peel & seed methanolic extracts and Chloroform extracts at doses of 100mg/kg and 150mg/kg were given orally in curative and preventive regimens over a period of 28 days. Cystone (750mg/kg) was taken as standard. An increase of serum calcium, serum oxalate, BUN, creatinine, uric acid, urine volume, urine PH and histopathological studies were observed in the calculi-induced group.
Results: It was found that Cucumis melo fruit peel and seed of chloroform extracts of 100mg/kg and 150mg/kg treated the hyperoxaluria, calcium, and uric acid, and renal function was improved. Furthermore, high serum levels of urea nitrogen, creatinine and uric acid were significantly ($P < 0.001$) reduced by the extract.
Conclusion: The fruit Cucumis melo can be consumed by patients with kidney problems and by healthy individuals.

INTRODUCTION

Urinary calculi are the third common issue in the urinary framework. Most calculi in the urinary framework emerge from a typical segment of pee, e.g. calcium oxalate speaking to up to 80% of dissected stones. In India, 12% of the populace is required to have urinary stones, out of which half may wind up with loss of kidneys or renal harm[1]. Melons are utilized by the antiquated individuals for some, medicinal purposes like diuretic, analegesic, mitigating, emetic etc. Geographically discovered generally in India, Persia, south Russia, and China. These incorporate smooth-cleaned assortments, for example, honeydew, Crenshaw, and casaba, and diverse got cultivars. Cucumis melo belongs to the family (Cucurbitaceae) likely local to Central Asia. The natural product is a generally expended whose major organically dynamic mixes are the vitamins C, genius vitamin A, and folic corrosive, the phenolic phytochemicals[2] and the dangerous cucurbitacins. Cucurbit seeds are promising substitutes for different nuts in drain refreshments. This is upheld by prove on the high protein exercises of urease, lipase, lipoxygenase[3], trypsin inhibitors and low action of β-amylase in musk melon. The seeds contain triterpenoid glucoside. The protein substance of seed supper is 49.93%. The seeds contain myristic corrosive, phosphates, galactane, lysine, citrulline, histidine, tryptophane, cysteine[4]. Kidney stones are reportedly affecting mankind since long time and have been one of the causes for renal failure[5][11][12]. As there is no single effective drug available for urolithiasis today, surgery is considered to be the best option especially when other alternatives fail. However it is expensive and not affordable for common man. Hence the natural drugs are considered to be next alternative.

Figure 1 Cucumis melo Fruit

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Figure 2 Cucumis melo Seed

MATERIALS & METHODS

Collection of Plant Material

The fruit of *Cucumis melo* was collected from the local market in the month of March. The fruit was authenticated by Dr. P.Satyanarayana Raju, Plant Taxonomist, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh.

Preparation of Plant Extract

The Cucumis melo fruit was thoroughly washed with water to remove the soil debris. The fruit peel and seeds were collected and were shade dried for 9-10 days until they become moisture free. Then the fruit peel and seeds were finely powdered. The finely powdered fruit peel and seed were subjected to defatting by petroleum ether at 60^{o}c. The defatted Cucumis fruit Peel and Seed are allowed for solvent extraction by using Chloroform & methanol by soxhlet extraction apparatus. The collected extract was distilled and the residue was preserved in refrigerator until further studies were carried out. The prepared extract was tested for phytochemical constituents present in Cucumis melo fruit Peel and Seed.

Drugs & Chemicals

Ethylene glycol (from Sigma Aldrich Chemicals Pvt Ltd , Bangalore, India). Cystone (from Himalaya Drug Company, Bangalore). Ethanol and chloroform for extractions (from Sigma Aldrich Chemicals Pvt Ltd, Bangalore, India). All the solvents used are for the extraction process are of laboratory grade. Demineralised water was purchased from local market.

Experimental Animals

Healthy adult male Wistar Albino rats (150-200gm) were obtained from the animal house of Mahaveer enterprises, Hyderabad, for study of antiurolithiatic activity. The animals were acclimatized to standard laboratory conditions (temperature: 25 ± 5°C), humidity (55 ± 5%) and maintained on 12-h light: 12-h dark cycle. They were provided with regular rat chow and drinking water ad libitum accordingly as per the instructions given by Institutional Animal Ethical Committee. (CPCSEA)[6]

Acute toxicity studies

The methanolic and chloroform extracts of cucumis melo were studied for acute oral toxicity according to the guideline No: 423 set by Organization of Economic Cooperation and Development (OECD)[7]. Two doses of 2000mg/kg (p.o) and 5000mg/kg (p.o) were tested on two groups containing five animals in each group for 14 days. The animals were monitered for mortality and general behaviour. Cucumis melo extract was safe upto 5000 mg/kg by oral route.

Experimental Procedure

Animals were divided into seven groups, each containing 6 animals. Group I served as normal control. Group II was fed with 0.75% ethylene glycol (EG) in water for induction of renal calculi. Group III received 0.75% ethylene glycol (EG) + standard drug cystone (750 mg/kg body weight) Group IV received 0.75% EG + Methanol extract of the peel at a dose of 150 mg/kg body weight. Group V received 0.75% EG + chloroform extract of the peel at a dose of 100 mg/kg body weight. Groups VI received 0.75% EG + Methanol extract of the seed at a dose of 150 mg/kg body weight. Groups VII received 0.75% EG + chloroform extract of the seed at a dose of 100 mg/kg body weight. The experiment was carried for 28 days. On 28th day urine and serum samples were collected and were analysed. Kidneys were isolated and histopathological studies are performed.

Statistical Analysis

The results of the study were expressed as mean± S.E. Data was analyzed by using one way analysis of variance test (ANOVA) followed by Dunnett's t-test for multiple comparisons. Values with $P < 0.05$ were considered as significant.

RESULTS

Table 1 Phytochemical Constituents of Cucumis melo fruit Peel and Seed.

Constituent	Cucumis melo peel	Cucumis melo seeds
Alkaloids	–	–
Carbohydrates	+	+
Glycosides	–	–
Steroids	+	+
Flavonoids	+	+
Saponins	–	–
Tannins	–	–
Terpenes	+	+
Phenols	+	+

Table 2 Effect of Cucumis Melo Fruit Peel & Seed Extract on Serum Calcium & Serum oxalate

Groups	CALCIUM(mg/ml)	OXALATE (mg/ml)
Normal	6.203 ± 0.174	5.754 ± 0.192
Ethylene Glycol (EG)	17.27 ± 1.347	16.32 ± 1.135
EG + Cystone	6.38 ± 0.212	5.928 ± 0.161
EG + CMMeP 150mg/kg	6.89 ± 0.273	6.035 ± 0.184
EG + CMChP 100mg/kg	6.75 ± 0.939**	6.098 ± 0.117**
EG + CMMeS 150mg/kg	6.47 ± 0.759**	5.936 ± 0.275**
EG + CMChS 100mg/kg	6.58 ± 0.585**	5.987 ± 0.287**

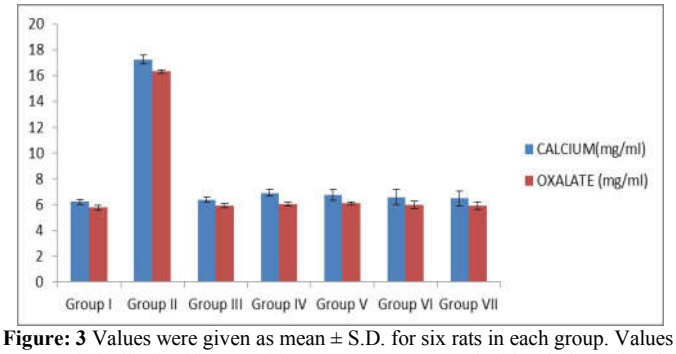


Figure: 3 Values were given as mean ± S.D. for six rats in each group. Values are statistically significant at **p < 0.001 as compared with Ethylene glycol group

Table 3 Effect of Cucumis Melo Fruit Peel & Seed Extract on Serum BUN

Groups	BUN(mg/ml)
Normal	23.37 ± 1.018
Ethylene Glycol (EG)	39.48 ± 1.47
EG + Cystone	28.74 ± 1.02
EG + CMMeP 150mg/kg	30.01 ± 1.173
EG + CMChP 100mg/kg	29.86 ± 1.139**
EG + CMMeS 150mg/kg	29.32 ± 1.12**
EG + CMChS 100mg/kg	29.64 ± 1.85**

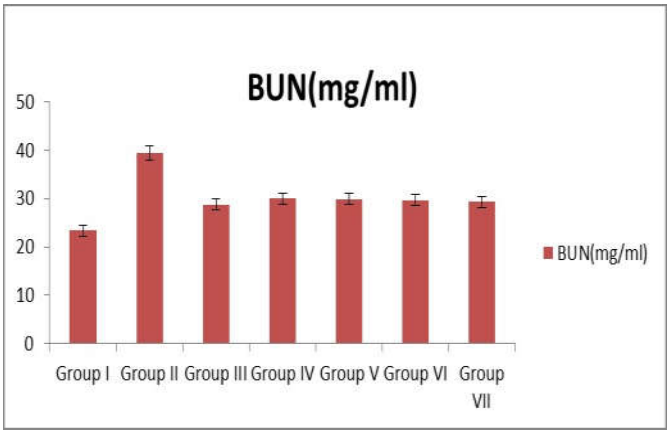


Figure: 4 Values were given as mean ± S.D. for six rats in each group. Values are statistically significant at **p < 0.001 as compared with Ethylene glycol group

Table 4 Effect of Cucumis Melo Fruit Peel & Seed Extract on Serum Creatinine

Groups	Creatinine (mg/ml)
Normal	0.47±0.05
Ethylene Glycol (EG)	0.99±0.035
EG + Cystone	0.514±0.04
EG + CMMeP 150mg/kg	0.67±0.084
EG + CMChP 100mg/kg	0.69±0.077**
EG + CMMeS 150mg/kg	0.63±0.075**
EG + CMChS 100mg/kg	0.66 ± 0.087**

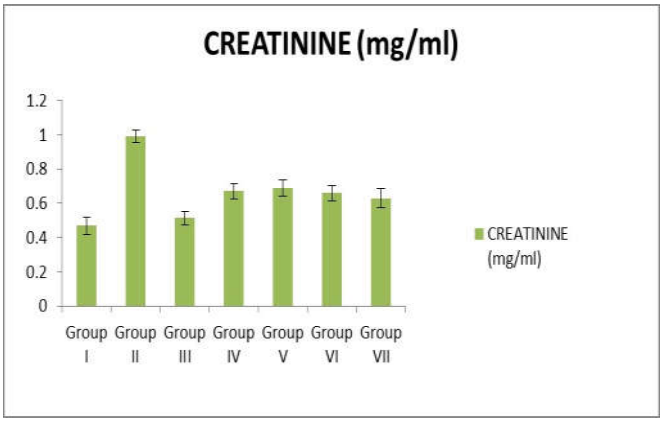


Figure: 5 Values were given as mean ± S.D. for six rats in each group. Values are statistically significant at **p < 0.001 as compared with Ethylene glycol group.

Table 5 Effect of Cucumis Melo Fruit Peel & Seed Extract on Uric acid

Groups	URIC ACID (mg/ml)
Group I	2.27 ± 0.06
Group II	4.67 ± 0.07
Group III	2.54 ± 0.09
Group IV	2.98 ± 0.084
Group V	2.96 ± 0.067**
Group VI	2.63 ± 0.027**
Group VII	2.79 ± 0.028**

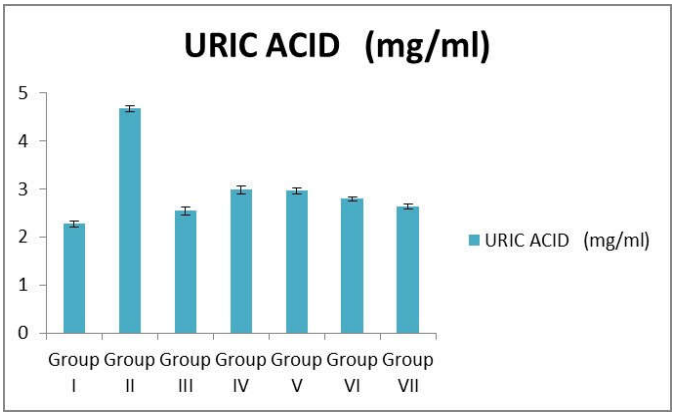
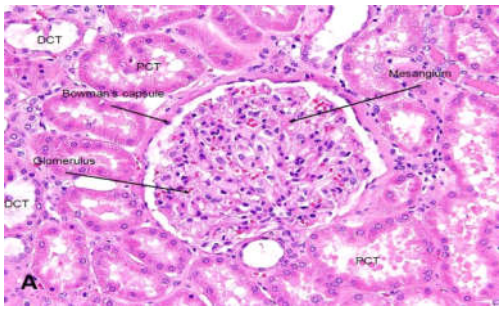
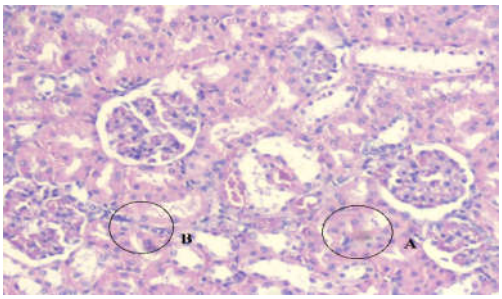


Figure: 6 Values were given as mean ± S.D. for six rats in each group. Values are statistically significant at **p < 0.001 as compared with Ethylene glycol group

Histopathology



Group I



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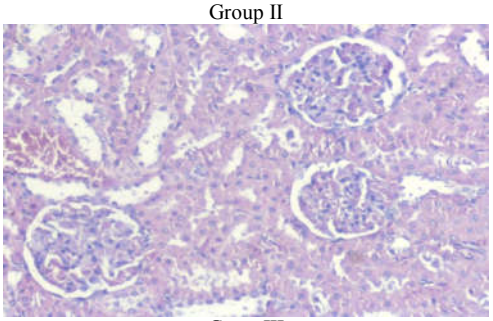
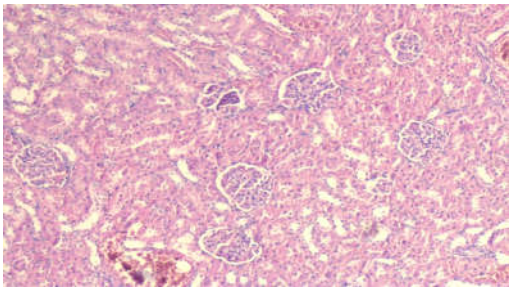


GroupIII



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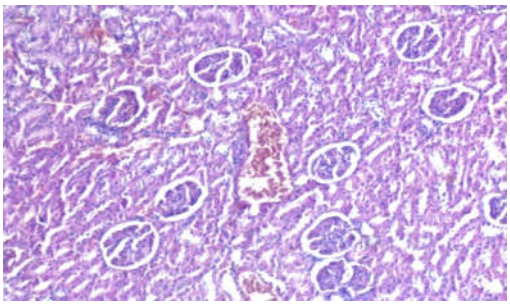
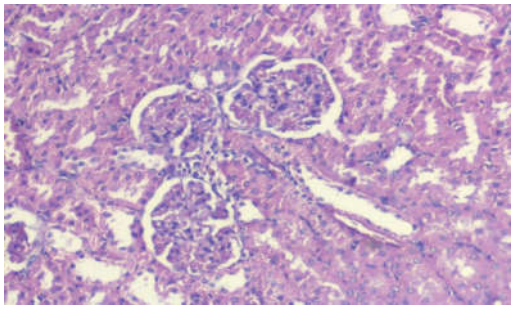
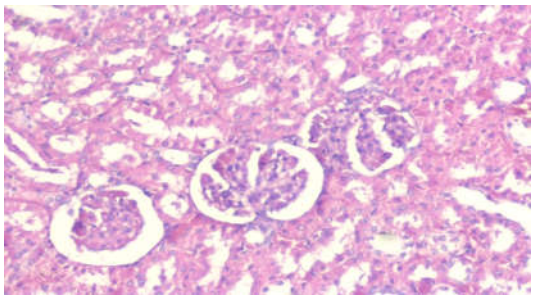


Group V



Group VI



Group-VII

Figure: 7 Histopathology of Kidney sections of Rats

Group- I: Normal - No degenerations and stone evidences found
Group- II: - EG treatment shows the glomerular degeneration and tubular congestion along with numerous microcrystals.
Group- III: - Cystone treatment shows the complete regeneration of all the nephrotic cells and with no evidence of stones
Group- IV: EG + CM Methanol Peel extract treated with 150 mg/kg has shown few evidences of crystal and tubular congestion
Group- V: EG + CM Methanol Seed extract treated with 150 mg/kg has shown no evidences of stones, but little tissue degeneration is found
Group- VI: EG + CM Chloroform Peel extract treated with 100 mg/kg has shown little evidence of stone and degeneration is very less
Group- VII: EG + CM Chloroform Seed extract treated with 100 mg/kg has shown no evidences of stones and degeneration. Complete regeneration of cells.

DISCUSSION AND CONCLUSION

The present study reveals that the seed and peel extracts of Cucucmis melo showed significant decrease in the excretion of serum calcium oxalate. The Chloroform extracts of 100mg/kg and 150mg/kg had no evidence of stones. When compared to methanolic seed 150mg extract, chloroform seed 100mg produced complete regeneration of renal tissues. Cucumis melo methanolic and chloroform peel extracts showed results almost equal to that of standard drug Cystone with less side effects.

As it was found from research reports that urinary super saturation is responsible for calculi formation, the urinary concentration of oxalate is found to be increased in ethylene glycol induced animals. This may be due to increased urinary retention and excretion of oxalate. In the present study it was found that urinary oxalate was increased in ethylene glycol induced urolithiasis rats whereas the excretion of oxalate was decreased in rats treated with Cucumis melo chloroform Peel and seed extract which may be due to the inhibition of formation of oxalate by the plant extract. This may also be due to the inhibition of the activity of oxalate oxidase enzyme which is reportedly responsible for the stone formation. Further study is required for isolation and evaluation of the chemical constituents which are present in the fruit and seed.

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