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Effect of *Cucumis melo* Seeds and Allopurinol on Litholytic and Renal Histological Profile in Male Rats

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ABSTRACT

Introduction: Kidney stones are the 3rd major health problem affecting the urological system. Industrial chemical ethylene glycol can generate calcium oxalate crystals in kidneys. Allopurinol has been used to treat the hyperuricemia but can be effective against calcium oxalate kidney stones too. Seeds of *Cucumis melo* were traditionally utilized as antioxidant and diuretic, so can be studied as litholytic agent against stones as well. **Aims & Objectives:** To assess and compare the litholytic effect of ethanolic extract of *Cucumis melo* seeds against ethylene glycol induced urolithiasis with allopurinol in rats. **Place and Duration of study:** This experimental study was conducted in Pharmacology Department of FPGMI, Lahore for the period of 2 months. **Material & Methods:** Four groups of 10 rats in each group were made. Calcium oxalate urinary stones were induced by 0.75% ethylene glycol for 28 days in male rats. Groups 1&2 served as negative and positive control while experimental groups 3&4 received Allopurinol 50mg/kg and *Cucumis melo* seed's ethanolic extract (EECMS) in dose of 400mg/kg from 15th day to 28th day respectively. At the end of study, kidney weight, urinary calcium, oxalate and phosphorus along with histological examination of kidneys were used as measuring criteria of litholytic effect of seeds of *Cucumis melo* and was compared to allopurinol. Data analysis was performed using SPSS 20.0 & presented in mean±s.d .Post-hoc Tukey's test and ANOVA were used for comparison between data groups **Results**: After 13 days of treatment experimental groups Groups 3 (Allopurinol 50mg/kg) and 4 (EECMS 400mg/kg) showed remarkable decreased mean urinary calcium, oxalate, phosphorus levels(mg/dl) and kidney weights(gm) of urinary calcium(10.55±1.53, 8.28±1.42), oxalate (18.3±0.78, 17.58±1.38), phosphorus (4.3±0.85, 3.59±1.49) and reduced kidney weights(1.43±0.22, 1.37±0.29) respectively(p value <0.001) as compared to diseased control group 2. Thus exhibiting *Cucumis melo* seed's ethanolic extract has better litholytic role than allopurinol which also proved to be efficacious. Histopathological examination of kidney also showed dissolution of calcium oxalate stones. **Conclusion:** Seeds of C*ucumis melo* extract have better litholytic effect on calcium oxalate kidney stones than allopurinol with improvement noted in the renal histological profile.

Key words: *EECMS, ethanolic extract of Cucumis melo seed;* Kidney stones, PO, per oral; litholytic.

INTRODUCTION

Urolithiasisis the stones formation in the urinary system.[1] It remains incurable with high recurrence rate affecting 12% population world wide.[2] So, it's a serious health issue in health care system. There are different types of stones but calcium oxalate and calcium phosphate constitute 75% of all urinary calculi.[3] Numerous pathological and chemical etiologies are involved in the pathogenesis of kidney stones including increase in super saturation of urine and accumulation of the crystals in the urinary tract. Then these crystals aggregate and grow in size to form the stones. The culprits of urolithiasis are found to be the hyperoxaluria and hypercalciurea which are responsible for ultimate stone formation and lipid peroxidation leading to kidney damage.[4]

In addition to this, raised level of phosphorus in urine further facilitate crystals of calcium oxalate to accumulate in urinary tract leading to enhanced damage to kidney.[5]

Patients with urolithiasis present with severe back flank pain, obstruction of urinary flow leading to hydronephrosis, infection, haemorrhage and finally renal failure[6]. Various medicines have been used for dissolving kidney stones. Allopurinol was utitilized previously for the treatment of hyperuricemia and uric acid stones but recently it can be commendably used for dissolving calcium oxalate stones by its antioxidant and reducing osteopontin expression capacity.[3,4,8,18] Carbonic anhydrase inhibitors and thiazide diuretics are prescribed for removal of acidic stones by alkalinization of urine and for the treatment of idiopathic hypercalciuria respectively.[7,8] All medicines are associated with serious adverse effects. Expensive therapeutic shock

waves may lead to acute kidney damage, reduced renal function and residual fragments of stone which increase the chances of infection and recurrence.[9] Thus, medical and surgical treatment of urolithiasis is expensive as well as associated with side effects[10] which have deviated humans to think upon and discover the beneficial effects of natural resources[11]. *Cucumis sativus, Ocimum gratisimum L, Macrotylo mauniflorum*, various herbs and plants are well known litholytic agents[4,10,12].

Cucumis melo is successfully used for different organ diseases including heart, gastrointestinal and genitourinary problems[11,13,15] as *Cucumis melo* seeds are rich sources of many diverse phytoactive compounds such as triterpenoids, flavonoids, alkaloids, SOD and others. These elements exhibit powerful diuretic, lithotriptic and litholytic activities.[12,13,16] Prevention of progression of crystallization can prevent the stone formation for sure.

This study was targeted to study influence of ethanolic extract of *Cucmis melo* seeds on calcium oxalate stones in kidneys induced by ethylene glycol and then it was compared to that of allopurinol in effectiveness.

MATERIALS AND METHODS

Study design: Experimental study

Place and Duration: Pharmacology Department, Shaikh Zayed Postgraduate Medical Institute, Lahore along with Postgraduate Medical Institute Lahore. 2 months.

Inclusion criteria: Male rats with body weight of 150-210 gram were included.

Exclusion criteria: Sick rats whose weight was less than 140gm were excluded

Sample size: 40 male rats, with weight of 150-210g were taken from the Animal House of University of Health Sciences. 10 rats were added four groups of 10 rats in each group were made.

Group 1: (Negative control) was control group and they were provided tap water for 28 days PO.

Group 2:(Positive control) consumed ethylene glycol 0.75% v/v for 28 days[5] PO.

Group 3: 0.75% Ethylene glycol was taken up by rats for first 15 days and 50mg/kg allopurinol was added from 15th day to 28th day[4] PO.

Group 4: 0.75% Ethylene glycol was taken up for first 15 days and 400mg/kg[13] *Cucumis melo* seed's ethanolic extract was added in water from 15th to 28th day PO.

Cucumis melo seed's ethanolic extract was prepared at Pakistan Council of Scientific and Industrial Research PCSIR.

Litholytic activity was assessed by measuring urinary calcium, oxalate and phosphorus levels together with weight and histopathological examination of kidneys in male experimental rats after getting consent from institutional review board.

Collection of urine: Rats were placed on the metal mesh 4 cm raised from bottom of cage. Urine was accumulated after 24 hours into bottom of cages on 28 day. These samples, after pouring a drop of concentrated HCL[4], were stored to measure calcium, oxalate and phosphorus contents.

Histopathology of kidney: Kidneys were taken out after dissection on 29 day and were embedded in paraffin wax to be stained with eosin and haematoxylin and for examination under microscope. Histopathological findings as index of urolithiasis are as follows: Focal epithelial damage, tubular dilation and presence of calcium oxalate crystals and casts within kidneys of rats[3,4].

Statistical analysis:

Data was evaluated by use of SPSS 20.0. Animal's calcium, oxalate and phosphate data was discussed by means of mean ± SD for four groups. Above mentioned parameters were compared between groups by utilizing post-hoc Tukey's test and ANOVA.

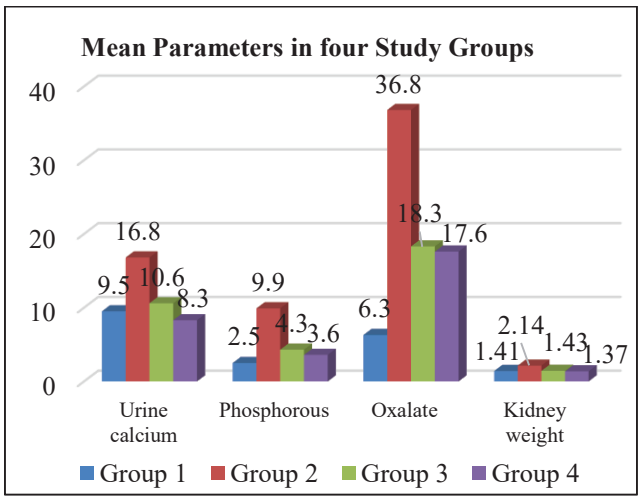
RESULTS

At the end of study, when compared, difference in means of urinary calcium (10.55±1.53, 8.28±1.42), oxalate (18.3±0.78, 3.59±1.49) and phosphorus (4.3±0.85, 3.59±1.49) in group 3 and 4 respectively were exceptionally significant with p-value <0.001(Table-1). Group 2 showed extensive raised values with p-value<0.001 when compared with group 1(Table-1). At the same time group 3and 4 showed striking decrease in levels of these crystal salts (Graph-1) using p-values <0.001 when compared to group 2 (Table-1). Urinary calcium, oxalate and phosphorus showed no difference having p-values 0.022, 0.935 and 0.500 respectively between the two experimental groups 3 and 4 (Table-1). The p-value of calcium, 0.022, means ethanolic extract has much better effect than allopurinol (Graph-1). Group 3 and 4 had noteworthy decreased mean kidneys weights 1.43±0.22, 1.37±0.29 respectively having p values <0.001 when compared to group 2 (Table-1). No marked discrepancy was noted with p-value 0.959 among 3 and 4 groups (Graph-1). Tubular dilation and damaged epithelium were absent in 100% animals of group 1 (Fig-1), 80% rats each in group 3 and 4, while it was present in 100% animals of

group 2 (Fig-2). Calcium oxalate crystals were absent in 70% animals in group 3 (Fig-3) and 80% in group 4 (Fig-4) having p values <0.001 when compared to group 2 with crystal buildup in 100% animals (Fig-2B).

Parameters	Group 1	Group 2	Group 3	Group 4
Oxalate (mg/dl)	6.3±0.62	36.78±5.02	18.3±0.78	17.58±1.38
Urinary calcium (mg/dl)	9.53±0.70#	16.79±2.29#	10.55±1.53#	8.28±1.42#
Phosphorus(mg/dl)	2.46±0.62*	9.86±1.11*	4.3±0.85*	3.59±1.49*
Kidney Weight(gm)	1.41±0.27*	2.14±0.22*	1.43±0.22*	1.37±0.29*

Table-1: All values are stated as mean±SEM of 6 rats(n=10)
#p<0.001 when compared to negative control
* p<0.001 when compared to positive control



Graph-1: Mean levels of kidney weights, urine calcium, phosphorus, oxalate levels for the albino rats in 4 study groups

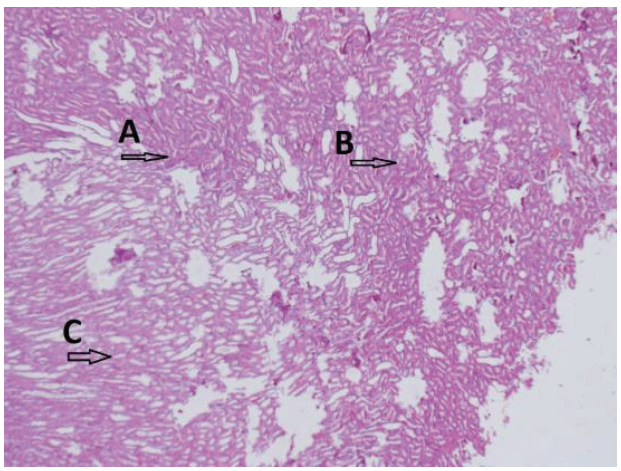


Fig-1: Histopathology of normal kidney(rat)under 4x A. Intact cortex and medulla B. Tubules with normal epithelium

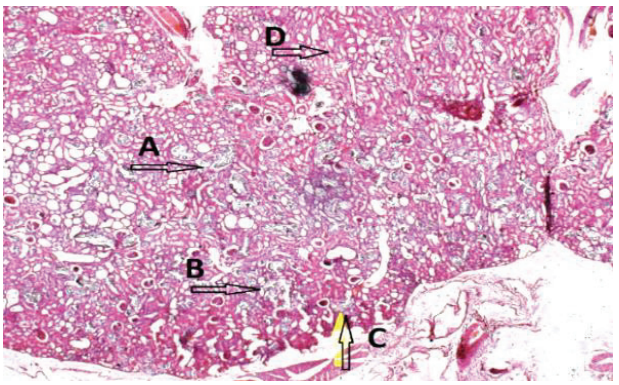


Fig-2A: Rat kidney (group 2) showing crystals of calcium oxalate and uric acid under 4x A, B. Tubules with accumulated crystals C, D. Damaged epithelium of tubule

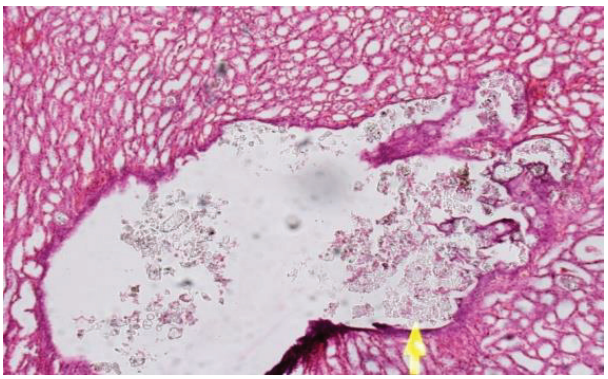


Fig-2B: Tubule dilated with crystal clusters (group 2) under 20X

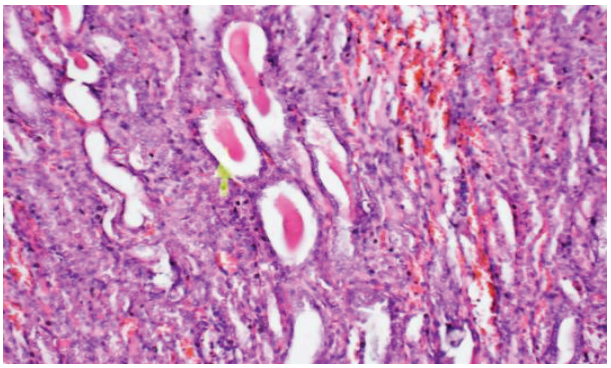


Fig-2C: Tubular damage, casts and haemorrhage (group 2) under 20x (yellow pointer)

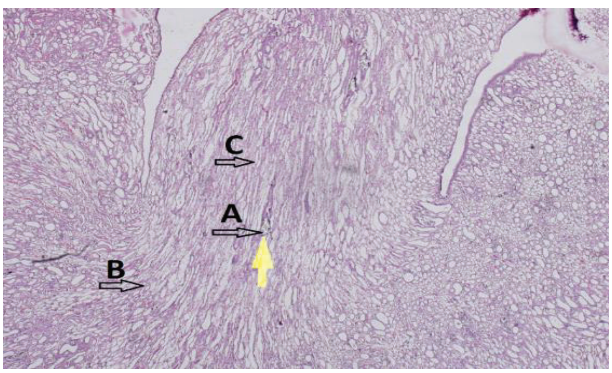


Fig-3: Histopathology of kidney(rat) received allopurinol (group 3) under 4x showing few crystals in few dilated tubules A. less no of crystals in few tubules B. Intact cortex and medulla junction C. Intact epithelium

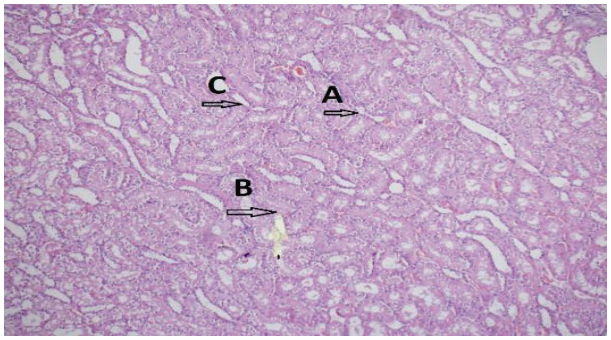


Fig-4: Rat kidney (group 4) received *Cucumis melo* seed ethanolic extract under 4x A.B. intact tubular epithelium C. Tubules clear of crystals

DISCUSSION

Urolithiasis is one of the common serious health issues with increasing prevalence all over the world. Despite many researches, it remains mostly incurable in allopathy because of increased recurrence rate and associated adverse effects of allopathy[1,2]. So varied investigations and laboratory techniques, are being done to elucidate the exact mechanisms and etiology of stone formation for better therapies to develop. Factors involved in the formation of stone might include super saturation of urine with insoluble materials which leads to crystals formation, nucleation and aggregation to form a stone[3]. The predominant stones are calcium oxalate or calcium phosphate[4].

This project aimed to resolve renal stones using potential cost effective phytotherapy.

The following study, judged antiurolithiatic activity by parameters urinary oxalate, calcium, phosphorus along with kidney weights and histopathological examination of kidneys.

Stones were induced in male rats as stone deposition in them was significantly higher in comparison to female rats[16,18].

Ethylene glycol produces hyperoxaluria which in turn facilitates calcium oxalate crystal deposition. This crystallization can destroy the renal epithelial cells directly[18, 19].

The rats in group 2 (positive control) receiving 0.75% ethylene glycol revealed marked rise in urinary calcium, oxalate, phosphorus and kidney weights(graph 1) having significant p value <0.001 due to deposition of calcium oxalate crystals and stones as shown in table-1.

The rats in group 4 receiving *Cucumis melo* seeds extract at dose of 400mg/kg for 13 days displayed significant decline in urine parameters (phosphorus, calcium, oxalate) and kidney weight with significant p value <0.001 when compared to group 2 (positive control) (Table-1). The reduction in kidney weights was the indicator of removal of stones and crystals from the kidneys showing their improved health. *Cucumis sativus*[4] have shown almost same results.

Cucumis sativus and *Cucumis melo* belong to the same genera with same phytochemicals [20,21].

Antioxidant, diuretic and anti-inflammatory properties of herbs are mainly because of phytochemicals such as triterpenoids, flavonoids, phenols, superoxide dismutase, alkaloids and saponins[10,16,17] which are also present within *Cucumis melo* seeds[11, 12, 13].

These phytochemicals upregulate and protect defensive mechanics by blunting formation of free radicals. However, the exact chemical compound responsible for litholytic activity is yet to be explored.

In group 3, 50mg/kg allopurinol for 13 days also declined urinary parameters and kidney weight with significant p value <0.001. These values are almost the same as that of *Cucumis melo* seed's ethanolic extract showing insignificant p values 0.500, 0.935 for urinary phosphorus and oxalate respectively (Table-1, Graph-1) proving that allopurinol and extract have same efficiency. Allopurinol has also similar effects in another studies due to its antioxidant effect and by reducing osteopontin expression that is key protein of calcium oxalate stone matrix[3,4,8,18].

Calcium in urine successfully declined when disparity was done to allopurinol with 0.022 p-value (Table-1, Graph-1). The effect of extract in reducing hypercalciuria is also evident in another study where *Cucumis melo* seeds have strong hypercitrituric effect resulting in reduced calcium excretion[5]. So, ethanolic extract of seeds of *Cucumis melo* is far healthier than allopurinol for calcium. This calcium lowering effect of seeds of *Cucumis melo* in urine could be effective way for the treatment of parathyroid disorders and bone diseases.

Histopathological examination of kidneys in group 2 showed calcium oxalate crystal clusters within dilated tubules and focal tubular damage (casts and haemorrhage) (Fig-2A, 2B, 2C). These changes were reversed in kidneys of rats in group 3 and 4 indicating the curative effect of the drug (allopurinol) (Fig-3) and ethanolic extract of seeds of *Cucumis melo* (Fig-4). Similar histopathological findings were noted by *Cucumis pepo*, identical genera, documenting the litholytic effect against calcium oxalate urolithiasis[14].

CONCLUSION

Hence it is concluded that seeds of C*ucumis melo* could be used as an excellent natural litholytic medicine for treatment of recurrent episodes of kidney stones and are better healthy and safe dietary replacement to drugs, for treatment of calcium oxalate stones and damaging changes caused by these stones. At the same time, allopurinol is also proven operational against calcium oxalate urolithiasis. However, the herbal medication is slow to act and time taking. Many patients don't comply with this.

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