



Protective Effect of *Cucumis melo linn* Seeds in Comparison with Allopurinol Against Oxidative Kidney Damage by Ethylene Glycol in Rats

¹Bushra Suhail, ²Saadia Shahzad Alam, ¹Khadija Mastoor, ²Mudassara Yaseen

¹Department of Pharmacology, University College of Medicine, University of Lahore

²Department of Pharmacology, Shaikh Zayed Medical Complex Lahore

ABSTRACT

Introduction: Ethylene glycol is a common commercial and industrial based chemical which is notorious for causing severe oxidative damage to different organs of body particularly the kidneys by deposition of oxalate stones. Allopurinol is effectively used in hyperuricemia induced renal oxidative stress and has shown potential benefits in treatment of calcium oxalate crystals as well. *Cucumis melo linn*, a member of cucurbitaceae family has well known antioxidant and diuretic properties so can be a phytoalternative nephroprotective agent for treatment of calcium oxalate stones. **Aims & Objectives:** To evaluate the nephroprotective effect of ethanolic extract of *Cucumis melo* seeds and its comparison with allopurinol against the renal damage due to deposition of kidney stones caused by ethylene glycol induced oxidative stress. **Place and duration of study:** PGMI Lahore, 2 months. **Material & Methods:** Nephrotoxicity was induced by 0.75% ethylene glycol in rats as proved by previous studies. 40 rats were equally divided in four groups. 400mg/dl of ethanolic extract of *Cucumis melo* seeds and 50mg/dl allopurinol were given orally in experimental groups from 15th day to 30th day. Protective role of Ethanolic extract of *Cucumis melo* seeds against nephrotoxicity due to ethylene glycol induced oxidative stress was assessed by measuring the body weight, blood urea nitrogen, creatinine and uric acid in the serum along with histopathological examination of kidneys in the experimental rats. It was compared to standard allopurinol which, by its antioxidant effect, reduces the oxidative damage and improves the renal function test and uric acid. **Results:** Results showed decreased levels of the blood urea nitrogen, creatinine and uric acid. Body weights were increased in experimental groups. All these results are significant with p value <0.001 showing nephroprotective effect of extract of *Cucumis melo* seeds and allopurinol. The results were further supported by histopathological examination of kidney. **Conclusion:** Ethanolic extract of *Cucumis melo* seeds has nephroprotective effect on ethylene glycol induced renal damage.

Key words: EECMS, ethanolic extract of *Cucumis melo seed*; RFTs, renal function test; BUN, blood urea nitrogen, Kidney stones, *Cucumis sativus*, Allopurinol, Uric Acid.

INTRODUCTION

Kidneys are the main organs in the body of humans and animals which are responsible for the excretion of waste products from the body^{1,2}. Chronic kidney disease is extremely serious health problem affecting people worldwide and has high incidence rate day by day. Urolithiasis is the complex chronic kidney disease that involves formation of stones in the urinary tract causing

changes in renal function test such as blood urea nitrogen and creatinine along with uric acid level which are considered as indicator to extent of renal damage^{3,4}. It is extremely painful condition leading to obstruction to urinary flow and ultimate renal failure if remains untreated⁵. Different chemicals can damage the kidneys to varying degree, ethylene glycol is one of those nephrotoxic agents. Previous studies have proved that administration of ethylene glycol for 14 days time period in rats can strongly alter the renal function tests and uric acid due to

deposition of stones in the urinary tract^{5,6}.

Clinically, allopurinol is a useful drug in chronic kidney disease associated with hyperuricemia, improves renal function due to its antioxidant properties by effectively decreasing the lipid peroxides^{7,8}. Various side effects of allopurinol have been documented such as gastrointestinal intolerance, peripheral neuritis, necrotizing vasculitis and rarely Steven Johnson's syndrome⁹. Diuretics such as carbonic anhydrase inhibitors and thiazides which have been successfully used for the treatment of chronic kidney disease have their own side effects¹⁰. The adverse effects of medicines and surgical procedures have motivated human to return to natural remedies with less to no side effects¹¹.

Various herbs and plants such as Lemon, *Cucumis sativus* and *Nigella sativa* L are known to have nephroprotective effect against ethylene glycol induced urolithiasis¹². *Cucumis melo* is also effective in treatment of hypertension, cancers, infections, peptic ulcer, constipation, menstrual disorders and anuria^{13,14}. *Cucumis melo* seeds contain active constituents like triterpenoids, alkaloids and flavonoids, having excellent diuretic and antioxidant activities^{14,15}. This study was designed to determine the protective effect of ethanolic extract of *Cucumis melo* seeds, against ethylene glycol induced oxidative stress and its effectiveness was compared to that of allopurinol.

MATERIAL AND METHODS

Place and duration: Department of Pharmacology and therapeutics, Shaikh Zayed Postgraduate Medical Institute, Lahore.

Sample size: 40 Male Albino rats, weighing 150-210g. They were divided into four groups.

Inclusion criteria: Male rats weighing 150-210 gram were included.

Exclusion criteria: Sick rats whose weight was less than 140gm were excluded Group A was negative control and were given tap water for 28 days.

Group B was positive control and were given ethylene glycol 0.75% v/v for 28 days.

Group C received ethylene glycol 0.75% for first 15 days and allopurinol 50mg/kg from 15th day to 28th day⁵.

Group D received ethylene glycol 0.75% for first 15 days and ethanolic extract of *Cucumis melo* seeds 400mg/kg¹⁴ from 15th day to 28th day. Nephro-

protective activity was assessed by measuring body weights and blood parameters such as the urea nitrogen, creatinine and uric acid along with histopathological examination of kidneys in rats.

Collection of blood:

On 29th day, 2ml of blood sample was taken by intracardiac puncture. Serum was analyzed for creatinine, urea and uric acid by spectrophotometer.

Histopathology of kidney:

The kidneys were isolated after dissecting rats and were dehydrated in ethanol and then embedded in paraffin wax for staining with haematoxylin and eosin for microscope examination. Histopathological changes as index of nephrotoxic kidney are as follows: Focal epithelial damage, eosinophilic cytoplasm, inflammation and thickening of basement membrane^{3,4}.

Statistical analysis

Data was analyzed by using SPSS 20.0. Data for animal uric acid, creatinine and BUN were described by using mean \pm SEM for all groups. Comparison for these parameters among groups was performed by using one way ANOVA and post-hoc Tukey's test as applied.

RESULTS

When compared, the difference in mean levels of BUN, creatinine, uric acid and body weights of rats were highly significant among groups with p-value <0.001. At pair wise comparison, it was observed that group 2, 3 and 4 had significantly higher levels as compared to group 1 with p-values <0.001. Similarly, the group 3 and 4 had significantly lower levels as compare to group 2 with p-values <0.001. There was no significant difference of BUN, creatinine, uric acid and body weights observed between the two experimental groups 3 and 4 with p-values 0.372, 0.353, 0.885, 0.999 respectively. Histopathological examination of kidneys in group 2 showed detached epithelium, eosinophilic cytoplasm, thickening of basement membrane all supportive of kidney damage (Fig-2A, 2B) whereas kidneys of group 3 and 4 showed normal renal tubular epithelium, cytoplasm, basement membrane and no inflammatory cells (Fig-3,4).

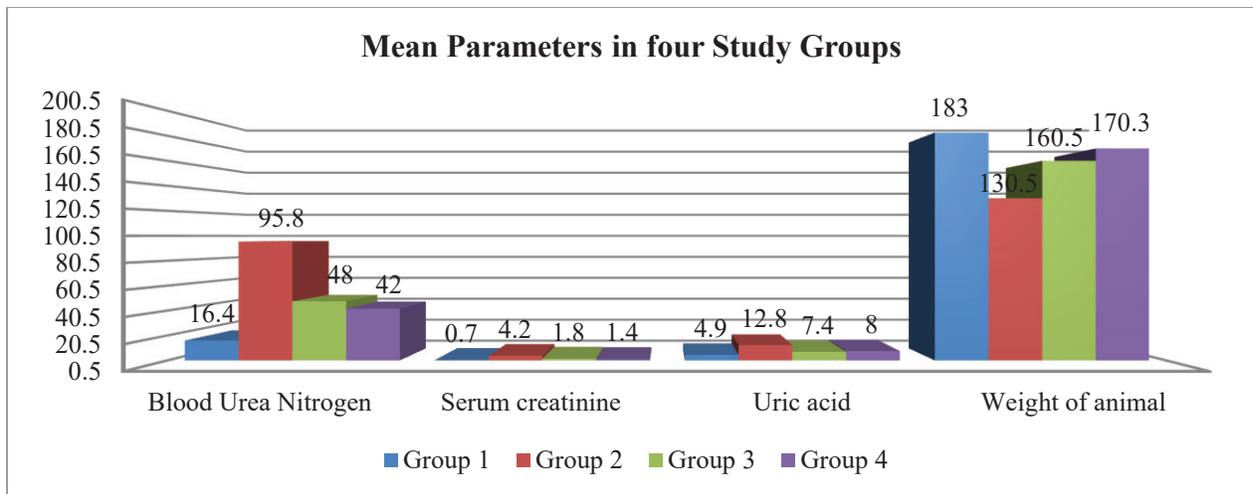


Fig-A: Mean blood parameters in mg/dl and body weights in gram of rats in four study groups.

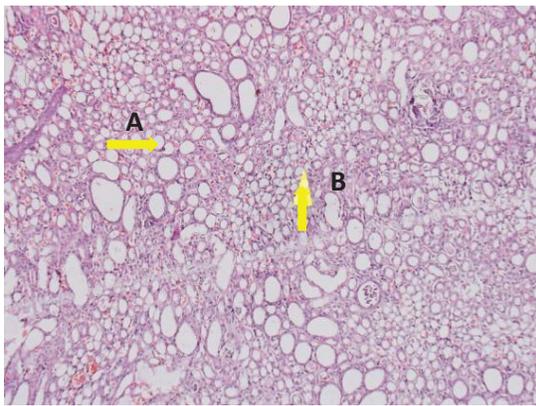


Fig-1: Histopathology of normal rat kidney under 4x showing A. Normal epithelium with cytoplasm B. Normal basement membrane

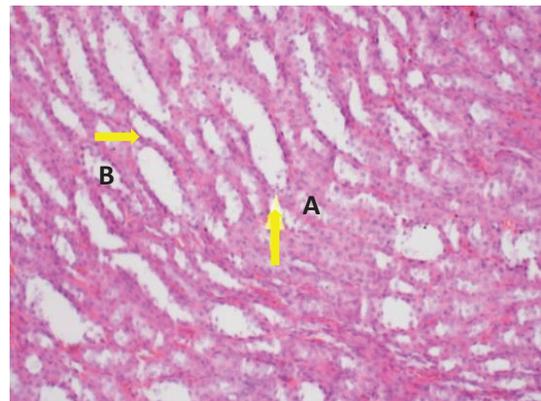


Fig-2B: A. Thickening of basement membrane B. Eosinophilic cytoplasm (group 2) under 20x (black pointer)

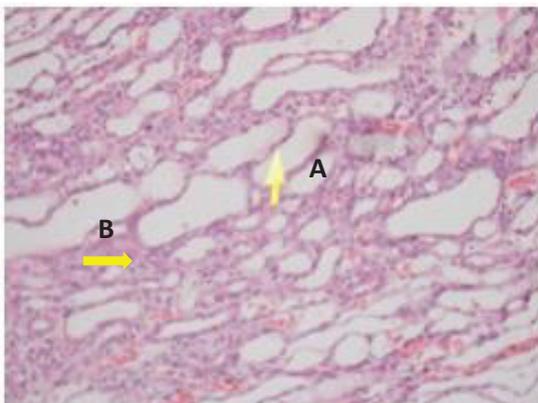


Fig-2A: A. Renal tubular epithelial damage B. Inflammatory cells (group 2) under 20x (black pointers)

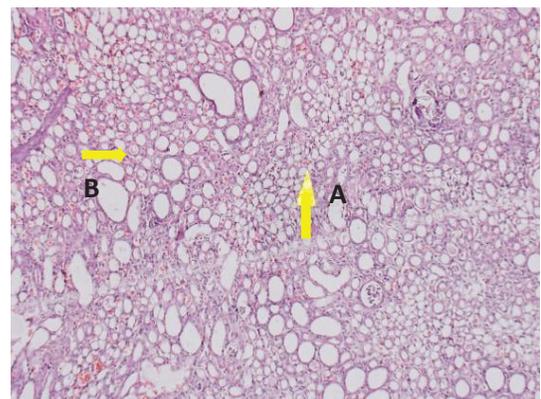


Fig-3: Histopathology of rat kidney (group 3) received allopurinol under 4x showing A. Intact epithelium of tubules B. No inflammatory cells

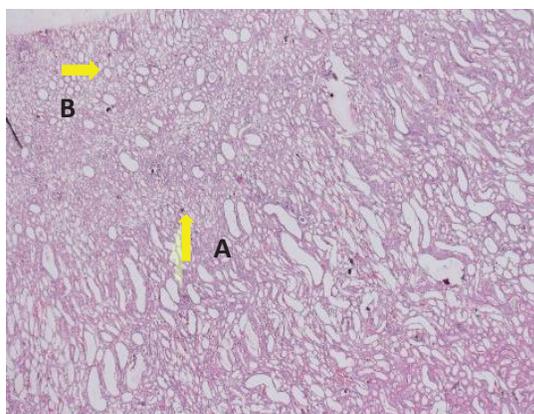


Fig-4: Rat kidney (group 4) that received ethanolic extract of *Cucumis melo* seeds showing A. Intact epithelium, normal cytoplasm and basement membrane B. No inflammatory calls under 4x

DISCUSSION

Ethylene glycol is oxidized in liver to glyoxylate and oxalate which causes metabolic acidosis and oxidative stress due to deposition of calcium oxalate crystals in kidneys⁴. Several etiopathological mechanisms may be involved but production of reactive oxygen species (ROS) in kidneys considered to be the major causative factor^{4,5,6}.

Reactive oxygen species can directly damage the renal epithelial cells and cause the release of pro-inflammatory cytokines and pain mediators which results in ultimate retention of nitrogenous waste products such as urea, creatinine and uric acid in blood^{11,13}.

The nephroprotective activity of different herbs was established due to combination of diuretic, anti-inflammatory and antioxidant effects which are found to be due to compounds such as triterpenoids, flavonoids, phenols, alkaloids, saponins^{16,17,20,21} which are present in abundance within ethanolic extract of *Cucumis melo* seeds^{15,18}.

On the basis of free radical scavenging²², anti-inflammatory¹¹ and powerful diuretic^{14,18} activities of *Cucumis melo* seeds, its nephroprotective activity against lipid peroxidation in kidneys due to ethylene glycol is established. In the present study, the rats in positive control group showed reduced body weight but marked elevation in blood urea nitrogen, creatinine and uric acid with significant p value <0.001.

Histopathological examination of kidneys showed detached epithelium, eosinophilic cytoplasm and thickening of basement membrane which are

indicative of kidney damage (Fig-2A, 2B). The rats administered ethanolic extract of *Cucumis melo* seeds in group 4 showed marked reduction in blood (BUN, creatinine, uric acid) parameters with p value <0.001 in comparison to group 3 at dose of 400mg/kg allopurinol. The body weights of rats in this group improved showing nephroprotective effect of extract of *Cucumis melo* seeds. Histopathological examination of kidneys showed normal renal tubular epithelium, cytoplasm, basement membrane and no inflammatory cells showing extract has regenerated epithelium (Fig-4). Similar results were also reported in another study with *Cucumis sativus*⁵ and *Cucumis pepo*¹⁷. These three belong to the same family and genera with same phytochemicals¹².

In another study, then nephroprotective activity of methanolic extract of *Cucumis melo* linn was established against renal damage induced by gentamycin in which the reduction in elevated blood urea nitrogen and serum creatinine along uric acid was noted with significant p value <0.001.

In group 3, allopurinol has also shown reduction in blood parameters with significant p value <0.001 and normal histology of rat kidneys with no epithelial damage (Fig-3). These results are comparable with those of ethanolic extract of *Cucumis melo* linn seeds with insignificant p values 0.372, 0.353, 0.885 for BUN, creatinine and uric acid respectively proving that extract and allopurinol have same efficacy.

CONCLUSION

On the basis of results, ethanolic extract of *Cucumis melo* seeds can be used as better alternative to certain allopathic drugs such as allopurinol, for chronic kidney disease to combat with nephrotoxic effects produced by recurrent episodes of kidney stones with raised levels of uric acid. Further research is required to elucidate the antiurolithiatic activity of the plant.

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The Authors:

Dr. Bushra Suhail,
Assistant professor
Pharmacology department,
University College of Medicine and Dentistry,
University of Lahore.

Prof. Saadia Shahzad Alam,
Head of Department Pharmacology
Shaikh Zayed Medical Complex Lahore.

Khadija Mastoor,
Associate Professor,
Pharmacology Department,
University College of Medicine and Dentistry,
University of Lahore.

Mudassara Yaseen,
Associate Professor,
Pharmacology Department,
Shaikh Zayed Medical Complex Lahore.

Corresponding Author:

Dr. Bushra Suhail,
Assistant professor
Pharmacology department,
University College of Medicine and Dentistry,
drbushrasher1@gmail.com