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

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**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 hyperuricimia 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.12 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 damage3,4. 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\textsuperscript{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\textsuperscript{7,8}. Various side effects of allopurinol have been documented such as gastrointestinal intolerance, peripheral neuritis, necrotizing vasculitis and rarely Steven Johnson’s syndrome\textsuperscript{9}. Diuretics such as carbonic anhydrase inhibitors and thiazides which have been successfully used for the treatment of chronic kidney disease have their own side effects\textsuperscript{10}. The adverse effects of medicines and surgical procedures have motivated human to return to natural remedies with less to no side effects\textsuperscript{11}. Various herbs and plants such as Lemon, \textit{Cucumis sativus} and \textit{Nigella sativa} L are known to have nephroprotective effect against ethylene glycol induced urolithiasis\textsuperscript{12}. \textit{Cucumis melo} is also effective in treatment of hypertension, cancers, infections, peptic ulcer, constipation, menstrual disorders and anuria\textsuperscript{13,14}. \textit{Cucumis melo} seeds contain active constituents like triterpenoids, alkaloids and flavonoids, having excellent diuretic and antioxidant activities\textsuperscript{14,15}. This study was designed to determine the protective effect of ethanolic extract of \textit{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 15\textsuperscript{th} day to 28\textsuperscript{th} day\textsuperscript{5}. Group D received ethylene glycol 0.75\% for first 15 days and ethanolic extract of \textit{Cucumis melo} seeds 400mg/kg\textsuperscript{14} from 15\textsuperscript{th} day to 28\textsuperscript{th} day. Nephroprotective 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\textsuperscript{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 ± 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).
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| Group | Blood Urea Nitrogen | Serum Creatinine | Uric Acid | Weight of Animal |
|-------|---------------------|------------------|----------|------------------|
| 1     | 16.4                | 0.7              | 4.9      | 183              |
| 2     | 48                  | 4.2              | 12.8     | 160.5            |
| 3     | 42                  | 1.8              | 7.4      | 170.3            |
| 4     | 42                  | 1.4              | 8        | 160.5            |

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

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

**Fig-3:** Histopathology of rat kidney (group 3) received allopurinol under 4x showing A. Intact epithelium of tubules B. No inflammatory cells
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 kidneys\(^4\). 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 scavenging\(^22\), anti-
inflammatory\(^11\) 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 phytochemicals\(^12\).

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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