

## Beneficial Effect of Artichoke Leaf Extract on Ethylene Glycol-Induced Urolithiasis in Rats

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### ABSTRACT

**Objective:** Urolithiasis is one of the most painful ailments of the urinary tract disorders found in humans. The aim of the present study was to investigate the anti-urolithiatic activity of artichoke leaf extract (ART) on ethylene glycol (EG)-induced urolithiasis in albino rats. **Methods:** urolithiasis was induced by adding ethylene glycol (0.75% v/v) to drinking water of rats for 28 consecutive days. Concurrently, ART (125, 250 and 500 mg/kg) were orally administrated either from the 1<sup>st</sup> day in the preventive regimen or from the 15<sup>th</sup> day in the curative regimen. A standard anti-urolithiatic drug, cystone (CST; 150 mg/kg; p.o.), was also used. **Results:** EG-induced UL was accompanied by an increase in the serum levels of uric acid, urea, creatinine and calcium with an increase in protein concentration in urine as indicators of renal damage. Moreover, induction of UL was associated with an elevated renal levels of lipid peroxides (measured as malondialdehyde; MDA) and reduced glutathione (GSH) as reliable indices of oxidative stress. In both regimens, administration of ART (125, 250 and 500 mg/kg; p.o.) restored the body weight, the kidney weight and the relative kidney weight. Moreover, ART decreased the serum levels of uric acid, urea, creatinine and calcium and also decreased the protein concentration in urine in a dose dependent manner. It also attenuated the kidney levels of MDA and GSH. **Conclusion:** ART has a protective effect on the kidney functions in EG-induced UL in rats probably due to its potent anti-oxidant property.

**Keywords:** ethylene glycol, urolithiasis, artichoke, anti-oxidant.

### INTRODUCTION

Kidney stone formation or urolithiasis (UL) is one of the most painful ailments of the urinary tract disorder. Urinary stone formation is a common disease that is increasing worldwide and appears even more pronounced in industrialized countries<sup>1</sup>. It is estimated to occur in approximately 12% of the world population, with recurrence rate is 30 to 40 % at 5 years as seen in observational study<sup>2</sup>. Formation of renal stones and their predominant chemical composition are age and gender dependent<sup>3</sup>. Urolithiasis is a consequence of an imbalance between promoters and inhibitors in the kidneys<sup>4</sup>. Though different kinds of stones have been identified, calcium stones are the most common in both human and rats<sup>5</sup>. The recurrence of UL represents a main issue for patients who formed one stone are more likely to form another. The standard drugs used to prevent UL are not effective in all patients, however most of which have adverse effects due to their long term use. Previous studies have shown that dietary modification or medication can significantly reduce the recurrence rate<sup>6</sup>. Artichoke (*Cynara scolymus*, Family *Asteraceae*) is an important component of the Mediterranean diet and it is rich in bioactive polyphenol compounds (mainly cynarin, luteolin and chlorogenic acid), dietary fibers, vitamins and minerals<sup>7</sup>. Traditionally, Artichoke leaves were used for the treatment and prevention of many diseases. Artichoke has been used to

treat dyspepsia mainly because of its choleric effect that is associated with increased bile formation<sup>8</sup>. Artichoke have been found to exhibit hepatoprotective activity<sup>9</sup>, lipid lowering property<sup>10,11</sup>, antioxidant effects<sup>9</sup> and reduce postprandial blood glucose<sup>12</sup> in man and experimental animals. Artichoke also produced protective effects against hepatocellular carcinoma both *in vitro* and *in vivo*<sup>7</sup>. Our study investigated the beneficial effects of artichoke leaf extract (ART) on ethylene glycol (EG)-induced UL in albino rats using two injection regimens. An anti-urolithiatic drug, cystone (CYS) was used as standard drug. Renal functions and oxidative stress were evaluated. Histopathological changes were also assessed.

### MATERIAL AND METHODS

#### Drugs and chemicals

Artichoke leaf extract (Mepaco) and ethylene glycol (Sigma–Aldrich) were used in the study. Ellman's reagent and thiobarbituric acid were purchased from Sigma–Aldrich (USA). All kits were purchased from Biodiagnostic Co. (Egypt). All other chemicals were of the highest analytical grade available.

#### Animals

Adult male albino Wistar rats, weighing 110–150 g, were used in this study. They were obtained from the Animal House Colony of the National Research Centre (Dokki, Giza, Egypt), and were housed under conventional

laboratory conditions throughout the study period. The animals were fed a standard rat pellet diet and allowed free access to water. The study was conducted in accordance with ethical procedures approved by the National Research Centre (Dokki, Giza, Egypt) – Medical Research Ethics Committee for the use of animal subjects.

#### *Induction of urolithiasis*

Urolithiasis was induced by adding EG (0.75% v/v) to drinking water of rats for 28 consecutive days<sup>13-15</sup>.

#### *Experimental design*

To assess the effects of ART, on EG-induced UL in albino rats using two injection regimens, two main sets of experiments were carried out depending on the duration of treatment. The first regimen, the protective regimen, ART was orally administered from the 1<sup>st</sup> day of the experiment in three dose levels while in the second regimen, the treatment regimen, ART was orally administered from the 15<sup>th</sup> day of the experiment in three dose levels. A standard anti-urolithiatic drug, CYS, was also used in both regimens. Rats were randomly allocated to one of four groups with 8 rats in each set of experiment as follows: One group received CYS (150 mg/kg; p.o.)<sup>13</sup> and three groups each received three dose levels of ART (125, 250 and 500 mg/dl), respectively<sup>7</sup>. In addition to two universal groups one serves as control negative group receiving normal drinking water and the other serves as control positive group where EG (0.75% v/v) is added to the drinking water of rats for 28 consecutive days. At the 28<sup>th</sup> day of the experiment, the rats were placed in metabolic cages, after twenty-four hours the urine was collected and the rats were weighed then anaesthetized with diethyl ether. Blood samples were collected and the serum was separated out to assay for renal biomarkers. The rats were then sacrificed by cervical dislocation and the kidneys were harvested, washed in normal saline, blotted with filter paper, weighed. Subsequently, one kidney was homogenized with an MPW-120 homogenizer (Bitlab), and the other was fixed in 10% formalin for histopathological examination.

#### *Determination of the protein in urine*

The method for determination of protein in urine was adopted by Daughaday, 1952<sup>16</sup> was measured colorimetrically at 700 nm.

#### *Determination of the serum renal function parameters*

The method for determination of serum creatinine was adopted by Schirmeister., 1964<sup>17</sup> and the resulting colored complex was measured colorimetrically at 520 nm. The method for determination of serum uric acid was adopted by Barham, 1972<sup>18</sup> and was measured colorimetrically at 510 nm. The method for determination of serum urea was adopted by Fawcett, 1960<sup>19</sup> and was measured colorimetrically at 550 nm. The method for determination of serum calcium was adopted by Gindler, 1972<sup>20</sup> was measured colorimetrically at 585 nm.

#### *Determination of the renal oxidative stress markers*

Renal levels of lipid peroxides were estimated as thiobarbituric acid-reactive substances (TBARS). The method of Mihara and Uchiyama (1978)<sup>21</sup> was adopted. The resulting pink-colored chromogen was extracted with butanol and the absorbance was measured at 532 nm.

Renal levels of reduced glutathione (GSH) were estimated using the methods of Beutler., (1963)<sup>22</sup>. This method depends on the fact that both protein and nonprotein thiol (-SH) groups (mainly GSH) react with Ellman's reagent [5,5'-dithiobis (2-nitrobenzoic acid)] to form a stable yellow color of 5-mercapto-2-nitrobenzoic acid, which can be measured colorimetrically at 412 nm.

#### *Histopathological examination*

Kidneys from the experimental rats sliced and fixed immediately in 10% formalin for at least 24 h. The specimens were then processed, embedded in paraffin, and cut into sections 5 mm thick. Sections were stained with hematoxylin and eosin for routine histopathological study.

#### *Statistical analysis*

Data are expressed as mean  $\pm$  SEM. We used one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test to judge the difference between the various groups. Values for  $P < 0.05$  were considered statistically significant for all of the experiments.

## **RESULTS**

#### *Effect of ART on kidney weight and relative kidney weight*

Induction of UL by adding EG (0.75% v/v) to drinking water of rats for 28 consecutive days was associated with a marked increase in their kidney weight and the relative kidney weight reaching about 160% and 213% of the normal value, respectively. In the preventive regimen, oral treatment of urolithiatic rats with ART (125, 250 and 500 mg/kg) for 28 days showed a reduction in the kidney weight reaching nearly the normal value and a reduction in the relative kidney weight reaching about 134%, 125% and 113% of the normal value, respectively, as compared to the urolithiatic group. However, similar treatment with CYS (150 mg/kg) normalizes the kidney weight and the relative kidney weight. In the treatment regimen, oral treatment of urolithiatic rats with ART (125mg/kg) for 14 days showed a reduction in the kidney weight reaching about 110% of the normal value, however, treatment with ART (250 and 500mg/kg) normalizes the kidney weight and a reduction in the relative kidney weight reaching about 140%, 115% and 121% of the normal value, respectively, as compared to the urolithiatic group. However, similar treatment with CYS (150 mg/kg) decreased the kidney weight and the relative kidney weight reaching about 123% and 126% of the normal value, respectively (Table 1).

#### *Effect of ART on protein in urine*

Moreover, induction of UL was associated with a marked increase in the protein in urine which reaching about 214 % of the normal value. In the preventive regimen, oral treatment of urolithiatic rats with ART (125, 250 and 500 mg/kg) for 28 days showed a reduction in the protein in urine reaching about 197%, 159% and 148% of the normal value, respectively, as compared to the urolithiatic rats. However, similar treatment with CYS (150 mg/kg) decreased protein in urine reaching about 144% of the normal value. In the treatment regimen, oral treatment of urolithiatic rats with ART (125, 250 and 500 mg/kg) for 14 days showed a reduction in the protein in urine reaching about 206%, 162% and 155% of the normal value, respectively, as compared to the urolithiatic rats. However,

Table 1: Effect of artichoke leaf extract on body weight, kidney weight and relative kidney weight of urolithiatic rats.

	Body weight (g)	Kidney weight (g)	Relative kidney weight (g/100body weight)
Normal	165±6.45	0.79±0.02	0.485±0.02
EG	124.16±2.74*	1.26±0.12*	1.034±0.11*
<i>Preventive regimen</i>			
EG+CYS	156.66±1.92 <sup>@</sup>	0.79±0.03 <sup>@</sup>	0.50±0.02 <sup>@</sup>
EG+ART (125 mg/kg)	128.33±3.04	0.82±0.01 <sup>@</sup>	0.65±0.02 <sup>@</sup>
EG+ART (250 mg/kg)	131.66±4.50	0.80±0.04 <sup>@</sup>	0.61±0.02 <sup>@</sup>
EG+ART (500 mg/kg)	143.33±5.22	0.79±0.02 <sup>@</sup>	0.55±0.02 <sup>@</sup>
<i>Treatment regimen</i>			
EG+CYS	161.66±12.28 <sup>@</sup>	0.97±0.05 <sup>@</sup>	0.61±0.01 <sup>@</sup>
EG+ART (125 mg/kg)	129.16±4.31	0.88±0.02 <sup>@</sup>	0.68±0.01 <sup>@</sup>
EG+ART (250 mg/kg)	132.5±4.20	0.74±0.01 <sup>@</sup>	0.56±0.02 <sup>@</sup>
EG+ART (500 mg/kg)	139.16±8.20	0.83±0.05 <sup>@</sup>	0.59±0.01 <sup>@</sup>

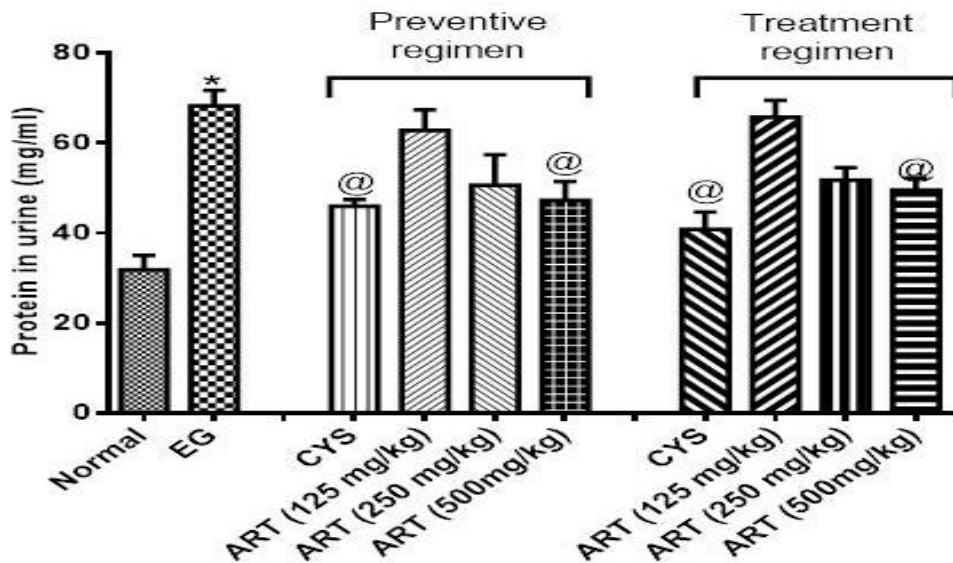


Figure 1: Effect of artichoke leaf extract on protein in urine of urolithiatic rats.

similar treatment with CYS (150 mg/kg) decreased protein in urine reaching about 147% of the normal value (Figure 1).

*Effect of ART on serum renal function parameters*

Induction of UL by adding EG (0.75% v/v) to drinking water of rats for 28 consecutive days was associated with a marked increase in their serum renal function parameters viz. serum levels of uric acid, urea, creatinine and calcium by 6, 4, 3 and 2 folds, respectively. In the preventive regimen, oral treatment of urolithiatic rats with ART (125, 250 mg/kg) for 28 days showed a reduction in the serum uric acid level reaching about 147% and 137% of the normal value, respectively; however, treatment with ART (500mg/kg) normalizes the serum uric acid level as compared to the urolithiatic group. Similar treatment with ART (125, 250 and 500 mg/kg) reduces in the serum urea level reaching about 260%, 164% and 118% of the normal value, respectively; and decreases in the serum creatinine level reaching about 262%, 266% and 229% of the normal value, respectively; and nearly normalizes the serum calcium level as compared to the urolithiatic group. However, similar treatment with CYS (150 mg/kg) decreased the serum levels of uric acid, urea, creatinine

and calcium reaching about 157%, 159%, 243% and 68% of the normal value, respectively. In the treatment regimen, oral treatment of urolithiatic rats with ART (125, 250 mg/kg) for 14 days showed a reduction in the serum uric acid level reaching about 200% and 147% of the normal value, respectively; however, treatment with ART (500mg/kg) normalizes the serum uric acid level as compared to the urolithiatic group. Similar treatment with ART (125, 250 and 500 mg/kg) reduces in the serum urea level reaching about 246%, 182% and 171% of the normal value, respectively; and decreases in the serum creatinine level reaching about 268%, 269% and 230% of the normal value, respectively; and decreases in the serum calcium level reaching about 148%, 138% and 128% of the normal value, respectively as compared to the urolithiatic group. However, similar treatment with CYS (150 mg/kg) decreased the serum levels of uric acid, urea, creatinine and calcium reaching about 125%, 168%, 251% and 94% of the normal value, respectively (Figure 2).

*Effect of ART on renal oxidative stress markers*

Induction of UL by adding EG (0.75% v/v) to drinking water of rats for 28 consecutive days was accompanied prominent change in the kidney oxidative stress markers

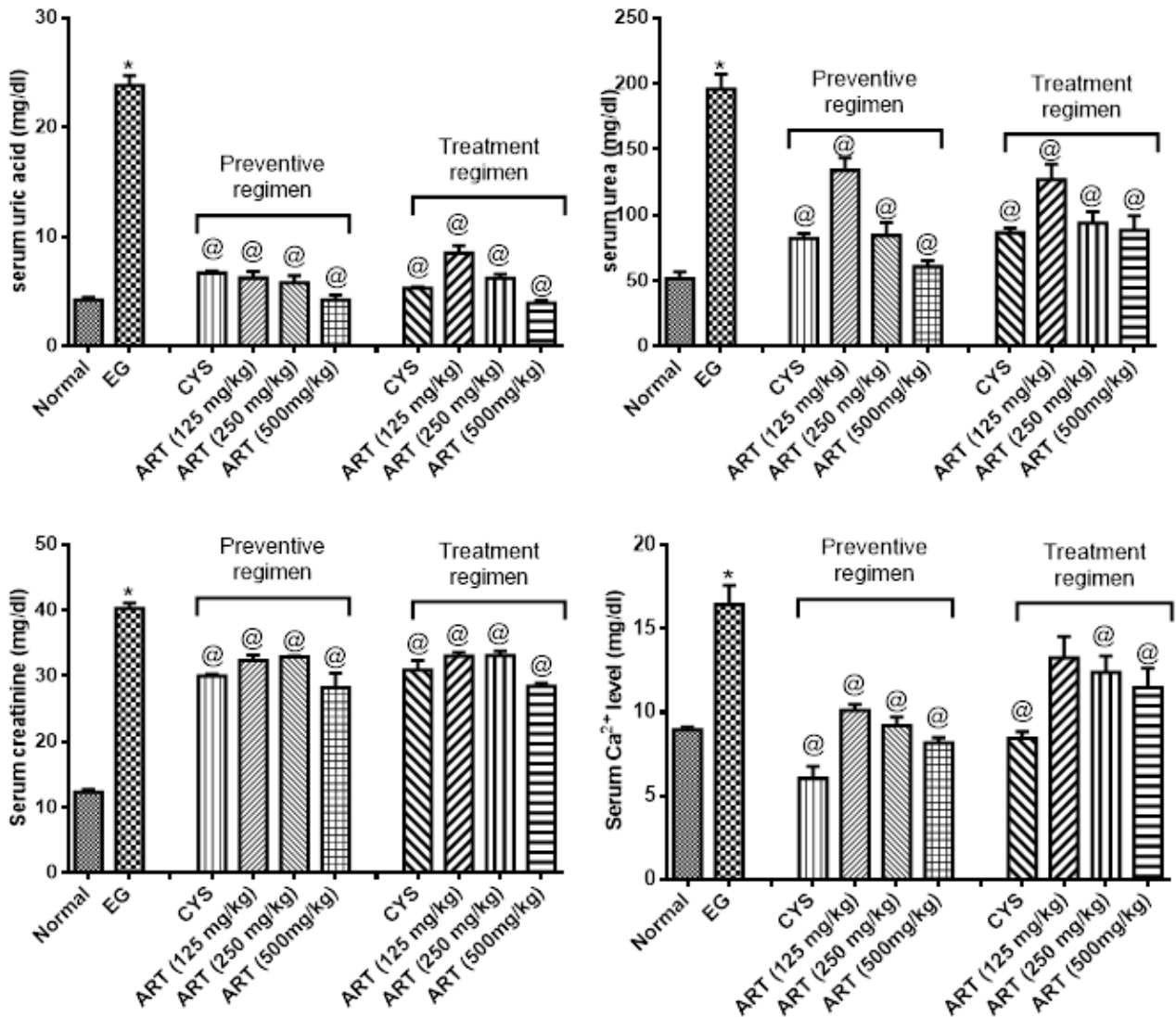


Figure 2: Effect of artichoke leaf extract on serum levels of uric acid (a), urea (b), creatinine (c) and calcium (d) of urolithiatic rats.

i.e. decrease in the kidney GSH level and an increase in the kidney MDA level reaching about 52% and 189% of the normal value, respectively. In the preventive regimen, oral treatment of urolithiatic rats with ART (125, 250 and 500 mg/kg) for 28 days nearly normalizes the kidney GSH level and showed a decrease in the kidney MDA level reaching about 140%, 137% and 118% of the normal value, respectively, as compared to the urolithiatic rats. However, similar treatment with CYS (150 mg/kg) normalizes the kidney GSH level and showed a decrease in the kidney MDA level reaching about 120% of the normal value. In the treatment regimen, oral treatment of urolithiatic rats with ART (125, 250 and 500 mg/kg) for 14 days nearly normalizes the kidney GSH level and showed a decrease in the kidney MDA level reaching about 152%, 151% and 133% of the normal value, respectively, as compared to the urolithiatic rats. However, similar treatment with CYS (150 mg/kg) normalizes the kidney GSH level and showed a decrease in the kidney MDA level reaching about 140% of the normal value (Figure 3).

*Effect of ART on histopathological examinations*

Induction of UL by adding EG (0.75% v/v) to drinking water of rats for 28 consecutive days was accompanied prominent change in the kidney histopathological examination showing oxalate crystals inside the lumen of tubules associated with tubular dilatation associated with vacuolar degeneration of tubular epithelium and showing multiple refractile oxalate crystals inside the lumen of renal tubules. However, oral treatment of urolithiatic rats with ART (125, 250 and 500 mg/kg) in both regimen showed no renal histopathological changes and with characters similar to normal control group (Figure 4).

**DISCUSSION**

Ethylene glycol is among many *in vivo* models developed to induce UL in rats. The toxicity of EG is due to its metabolic pathway which involves an alkylation reaction, producing the formaldehyde and aldehyde. EG can be further metabolized to glycolate by alcohol/aldehyde dehydrogenase enzymes<sup>23</sup>. In accordance with other studies, induction of UL by adding EG (0.75% v/v) to drinking water of rats for 28 consecutive days was

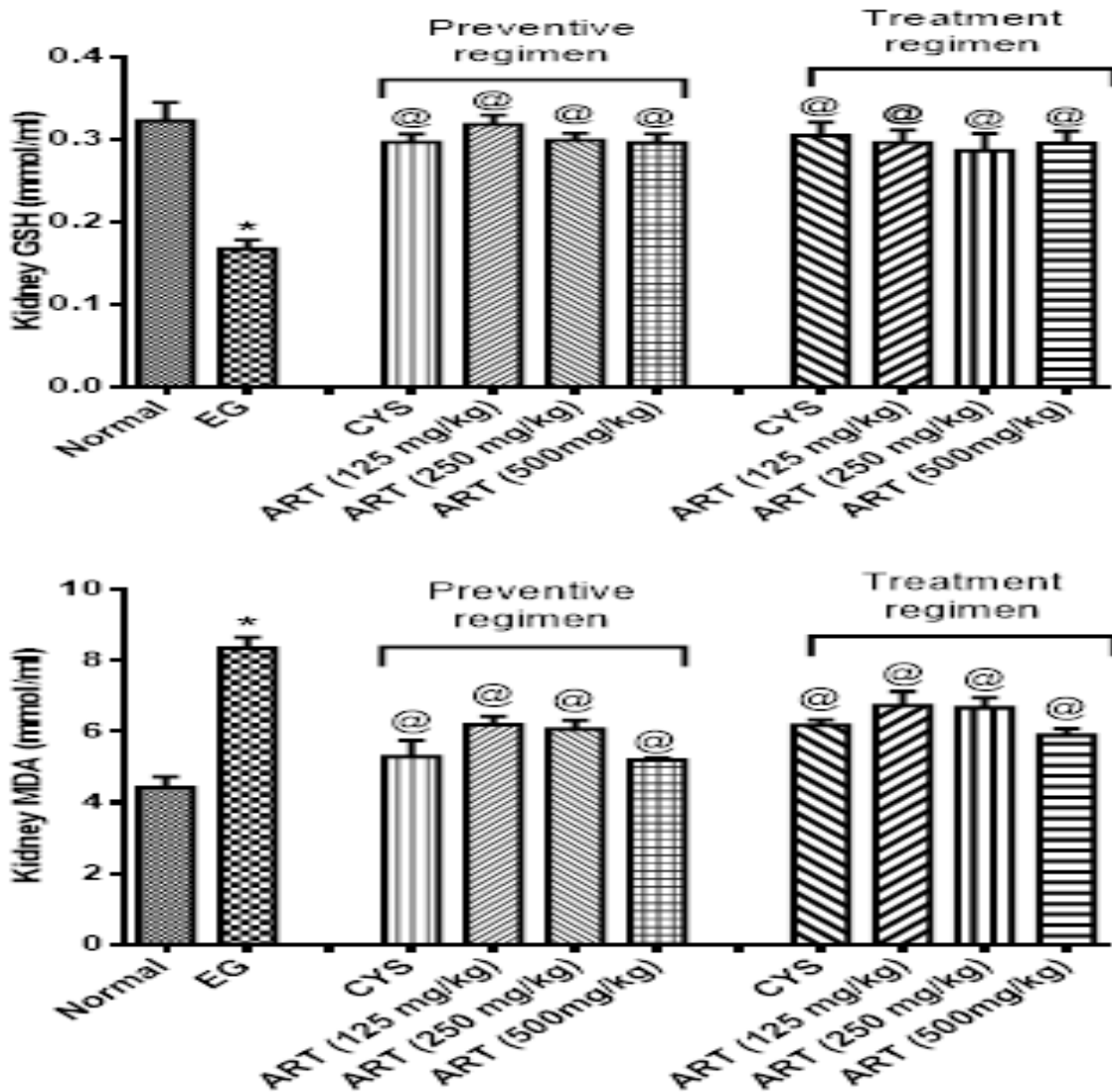


Figure 3: Effect of artichoke leaf extract on kidney levels of reduced glutathione (a) and malondialdehyde (b) of urolithiatic rats.

associated with a marked increase in their serum kidney function parameters *viz.* serum levels of uric acid, urea, creatinine and calcium and a prominent change in the kidney oxidative stress markers i.e. decrease in the kidney GSH level and an increase in the kidney MDA level<sup>14,24</sup>. The basic mechanism behind EG-induced UL is increasing urine volume and lowering urinary pH, along with hypercalciuria and hyperoxaluria leading to calcium oxalate crystal formation<sup>25</sup>. Deposition of calcium oxalate crystal is accompanied by severe oxidative stress to renal tissue<sup>26, 15</sup> leading to lipid peroxidation of membranes by generation of reactive oxygen species (ROS) like hydroxyl, superoxide ions<sup>27</sup> thus decreasing the activity of anti-oxidant enzymes in the kidneys of untreated rats<sup>14</sup>. It has been observed that anti-oxidant therapy prevents EG-induced renal calcium oxalate crystal deposition in rats which protect against nephrolithiasis<sup>28</sup>. In the current study, oral treatment of urolithiatic rats with ART (125, 250 and 500 mg/kg) in both regimens attenuated the serum kidney function parameters as well as kidney oxidative stress markers. Moreover, kidneys isolated from rats

treated with ART showed no histopathological changes and clear from crystals inside the lumen of renal tubules. This is attributed to the antioxidant property of ART may have contributed in restoration of EG-induced renal dysfunction. Artichoke leaf extract has antioxidant properties<sup>29</sup>. The protective activity of ART could be attributed to its constituents of many bioactive polyphenolic antioxidant compounds, mainly cynarin, luteolin and chlorogenic acids<sup>12</sup> via increasing GSH levels and decreasing lipid peroxidation. As far as we know this study is the first to evaluate the effect of ART on EG-induced UL, a state of with elevated renal oxidative stress. However, there are other studies that evaluated its beneficial effect in oxidative stress-induced hepatotoxicity<sup>9,12,30,31</sup>. Moreover, ART protected cultured rat hepatocytes against hydroperoxide induced oxidative stress<sup>32</sup>. ART also inhibited L low density lipoprotein (LDL) oxidation<sup>10</sup> and reduced the release of intracellular ROS by oxidized LDL in cultured endothelial cells and monocytes<sup>33</sup>. Similarly, CYS therapy provides protection against hyperoxaluria-induced oxidative stress and



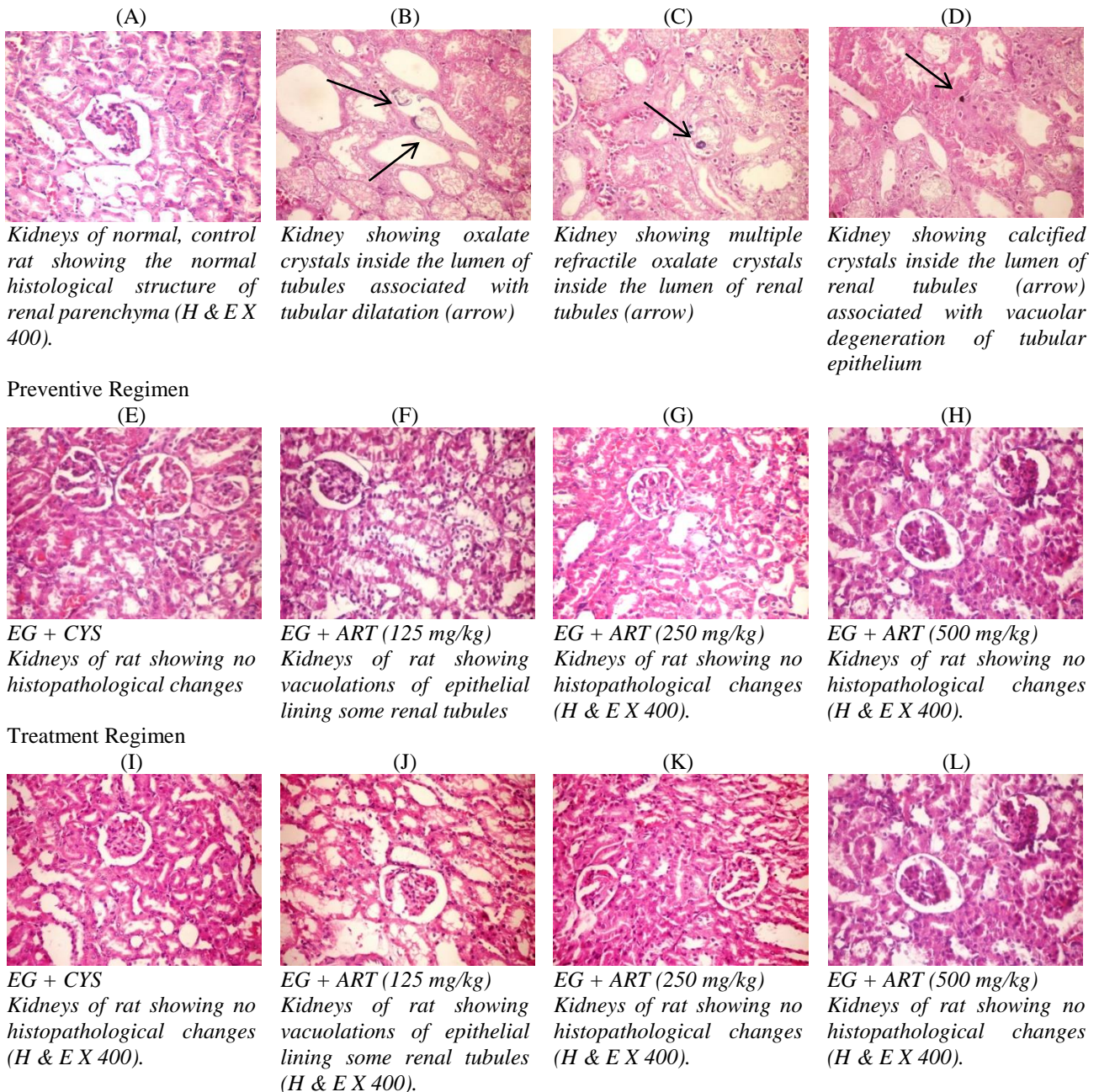


Figure 4: Effect of artichoke leaf extract on histopathological examination of the kidney isolated from ethylene glycol-treated rats.

calcium oxalate crystal deposition by improving renal tissue antioxidant status and diuresis (Bodakhe *et al.*, 2013)<sup>14</sup>. Urolithiasis induced by EG was associated with a marked increase in the kidney weight and the relative kidney weight for 28 consecutive days. This is probably due to hypertrophy of renal papilla, inflammation and fluid accumulation (Mandavia *et al.*, 2013)<sup>23</sup>. The kidneys isolated from EG-treated rats showed tubular dilatation in histopathological examination. Restoration of renal function is also associated with improvement in kidney weight in ART treated rats in prophylactic group. Positive effect on kidney weight, renal function and histopathological imaging suggests its nephroprotective effect in renal calculi. From all these findings, we conclude that ART is useful as a preventive and therapeutic agent

against the formation of oxalate kidney stones. Urolithiasis was induced by adding EG (0.75% v/v) to drinking water of rats for 28 consecutive days. Concurrently, ART (125, 250 and 500 mg/kg) were orally administrated either from the 1<sup>st</sup> day in the preventive regimen or from the 15<sup>th</sup> day in the curative regimen. Rats were weighed then sacrificed and the kidneys were isolated, weighed and the relative kidney weights were calculated. Results are expressed as means±SEM (n =6–10). \*Significant difference from normal rats P < 0.05. @ Significant difference from urolithiatic rats P < 0.05. Urolithiasis was induced by adding EG (0.75% v/v) to drinking water of rats for 28 consecutive days. Concurrently, ART (125, 250 and 500 mg/kg) were orally administrated either from the 1<sup>st</sup> day in the preventive

regimen or from the 15<sup>th</sup> day in the curative regimen. Rats were placed in metabolic cages and the urine is collected for determination of its protein content. Results are expressed as means±SEM (n =6–10). \*Significant difference from normal rats P < 0.05. @ Significant difference from urolithiatic rats P < 0.05. Urolithiasis was induced by adding EG (0.75% v/v) to drinking water of rats for 28 consecutive days. Concurrently, ART (125, 250 and 500 mg/kg) were orally administered either from the 1<sup>st</sup> day in the preventive regimen or from the 15<sup>th</sup> day in the curative regimen. Blood samples were collected and the sera were separated for determination of the levels of uric acid, urea, creatinine and calcium. Results are expressed as means±SEM (n =6–10). \*Significant difference from normal rats P < 0.05. @ Significant difference from urolithiatic rats P < 0.05.

#### *malondialdehyde (b) of urolithiatic rats.*

Urolithiasis was induced by adding EG (0.75% v/v) to drinking water of rats for 28 consecutive days. Concurrently, ART (125, 250 and 500 mg/kg) were orally administered either from the 1<sup>st</sup> day in the preventive regimen or from the 15<sup>th</sup> day in the curative regimen. Rats were sacrificed and the kidneys were isolated and homogenized for the determination of the kidney levels of GSH and MDA. Results are expressed as means±SEM (n =6–10). \*Significant difference from normal rats P < 0.05. @ Significant difference from urolithiatic rats P < 0.05. Urolithiasis was induced by adding EG (0.75% v/v) to drinking water of rats for 28 consecutive days. Concurrently, ART (125, 250 and 500 mg/kg) were orally administered either from the 1<sup>st</sup> day in the preventive regimen or from the 15<sup>th</sup> day in the curative regimen. Rats were sacrificed and the kidneys were isolated and Kidneys from the experimental rats sliced and fixed immediately in 10% formalin for at least 24 h. The specimens were then processed and sections were stained with hematoxylin and eosin for histopathological study.

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