Protective effect of magnesium lactate gluconate and *Garcinia cambogia* fruit extract in experimentally induced renal calculi in rats

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**ABSTRACT**

**Aim:** Antiurolithiatic activity of magnesium lactate gluconate (MLG) and aqueous extract of *Garcinia cambogia* (GC) fruit was studied. **Materials and Methods:** The study was performed during December 2016 to April 2017. Urolithiasis was induced in male Wistar rats by administration of 0.75% v/v ethylene glycol for 21 days. From 8th day onward, intervention with MLG (200 and 400 mg/kg b.w.) and GC (100 and 200 mg/kg b.w.) was started. At the end of the treatment period, biochemical parameters affecting renal stone formation were estimated in the serum, urine, kidney homogenate, and histopathology of harvested kidneys was performed. **Results:** From *in vivo* evaluation, it was observed that MLG 400 mg/kg b.w., GC 100 mg/kg b.w., and GC 200 mg/kg b.w. significantly reduced nitrogenous waste products in serum (blood urea nitrogen, creatinine, and uric acid) as well as calculogenic promoters in urine (phosphate and oxalate) and kidney homogenate (calcium, phosphate, and oxalate) when compared to disease control animals. The MLG 200 and MLG 400 were ineffective in restoring superoxide dismutase (SOD) and catalase (CAT) enzyme activity, whereas GC 100, GC 200, and Cystone® 400 mg/kg b.w. significantly elevated SOD and CAT enzymes in urolithic rat kidney. **Conclusions:** MLG and GC extracts are capable of preventing calcium oxalate (CaOx) crystal formation and subsequent deposition in renal tubules. The principle mechanism underlying nephroprotective effect of test drugs might be attributed to their calcium ion chelating ability and CaOx crystallization inhibitory activity. It is further asserted that GC was more potent than MLG in overall kidney protection by virtue of its antioxidant potential.

**KEY WORDS:** Ethylene glycol, *Garcinia cambogia*, hyperoxaluria, kidney stone, magnesium lactate gluconate, urolithiasis

**INTRODUCTION**

Urolithiasis, also known as nephrolithiasis (stone formation in kidneys), is a common disease with an increasing prevalence irrespective of geographic areas affecting approximately 5-12% of world population and has a significant impact on patient’s quality of life [1,2]. Pathobiology of kidney stone formation is multifactorial which includes heredity, diet, metabolic abnormalities such as hypercalcemia, hyperuricosuria, hyperoxaluria, and infection as causative factors [3,4]. More than 85% of the stones in human are comprised of calcium oxalate (CaOx) and calcium phosphate (CaP), and remainders are made of uric acid, cystine, and struvite [5]. It is postulated that urinary supersaturation as a result of low urine volume alone may increase the urinary concentration of calcium salts leading to the formation of urinary calcium crystals [6,7].

Current prophylactic treatments of CaOx kidney stone include increasing water intake, dietary restrictions, and urinary alkalizing and calcium chelating agents (sodium bicarbonate, potassium citrate, and sodium citrate) which collectively reduce CaOx supersaturation in urine. Thiazide diuretics (hydrochlorothiazide) and allopurinol (in patients with idiopathic CaOx stones in hyperuricosuria) are also being used in the management of CaOx stone disease [8]. However, it is also reported that the risk of recurrence is 40% at 5 years and 75% at 20 years after passage of a first stone [9].

Although these treatments can be effective as prophylaxis, they may have little role in preventing incidences of stone recurrence. In addition, many of the current therapies have significant adverse consequences. For instance, thiazides decrease urinary calcium excretion but may lead to hypocalcemia which in turn cause hypocitraturia and promote stone formation. Similarly, sodium bicarbonate therapy may precipitate CaOx (due to sodium overload) or CaP stone (due to alkaline pH) [8]. This implies that there is a greater need to develop more effective drugs for treating stone disease with fewer or no complications. Therefore, the present investigation is undertaken to study
antiurolithiatic activity of some alternative therapeutic agents, namely, magnesium lactate gluconate (MLG) and Garcinia cambogia (GC) fruit aqueous extract.

A literature survey indicates that MLG is freely water-soluble and low-tasting source of magnesium in beverages, food, and nutritional supplement application. It further states that 7.5 g of MLG provides 100% of recommended daily allowance for magnesium [10]. Thus far MLG is not explored for any therapeutic effects. However, magnesium is an inhibitor of stone formation and can complex with renal oxalate [3]. It is presumed that interaction of MLG with CaOx stone may produce calcium lactate gluconate (CLG) which has a very high water solubility compared to other calcium salts (400 g/L) [11]. Another investigational agent - GC fruit aqueous extract is reported to contain a high concentration of hydroxycitric acid (HCA) [12]. It has been demonstrated that lethal dose 50% of hydroxycitrate in male and female albino rats was more than 5000 mg/kg in oral acute toxicity study [13]. Despite its high HCA content, GC has not been scientifically validated for its antiurolithiastic potential.

In vitro studies performed by us revealed that MLG and GC caused inhibition of CaOx nucleation by 74.18 ± 8.97% (at 800 µg/ml, pH 10) and 61.25 ± 8.31% (at 200 µg/ml, pH 10), respectively. Moreover, MLG and GC showed inhibition of crystal aggregation by 62.77 ± 11.83% (at 800 µg/ml, pH 10) and 73.51 ± 5.14% (at 200 µg/ml, pH 10), respectively, suggesting that both drugs are acting through different mechanisms [14]. From in vitro results, it was interpreted that both test drugs were found to be equi-efficacious at different concentrations. GC is effective at low doses, and beyond 200 µg/ml, it did not produce any significant benefits over previous doses tested. Contrary to that, MLG showed dose-dependent action over a range of concentrations (50-800 µg/ml). However, it required higher concentration (800 µg/ml) to produce nearly same effect as GC [14]. Based on above scientific information, MLG (at a dose of 200 and 400 mg/kg b.w.) and GC (at 100 and 200 mg/kg b.w.) were investigated for their antiurolithiastic potential in ethylene glycol (EG)- and ammonium chloride (AC)-induced urolithiasis in rats.

MATERIALS AND METHODS

Materials and Instruments

GC fruit aqueous extract containing 60% (-) HCA (Panacea Phytoextracts, Ahmedabad, India) and Cystone® (The Himalaya Drug Company, Bengaluru, India) was purchased from commercial sources. MLG was obtained as a gift sample from Gujwell Biotech (P) Ltd., Siliguri, India. Biochemical estimation kits for calcium, phosphate, blood urea nitrogen (BUN), creatinine, and uric acid were purchased from Span Diagnostics Ltd., Surat, India. All chemicals and reagents were of analytical grade and procured from approved chemical suppliers. Equipment such as metabolic cages (INCO, Ambala, India), cooling centrifuge (Remi Instruments Division, Vasai, India), semiauto-chemistry analyzer (Rayto Life and Analytical Science Co. Ltd., Shenzhen, China), and ultraviolet spectrophotometer (UV 1800, Shimadzu, Japan) were used in the study.

Animals

The in vivo study was performed on 7-8-week-old male Wistar rats weighing 150-200 g. Animals were housed in clean polypropylene cages (3 per cage) under standard environmental conditions (12/12 h light/dark cycles at 22 ± 3°C and 50 ± 5% relative humidity). The animals were acclimatized to the laboratory conditions for a week before the experiments and fed with standard pellet diets (Keval Sales Corporation, Baroda, India) and water ad libitum. Animals were maintained in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals’ Guidelines, Ministry of Environment and Forest, India, for the care and use of laboratory animals. The experimental protocol was reviewed and approved by Institutional Animal Ethics Committee (Ref. no. IAEC18/RPJ19/2016).

Experimental Design

The study was performed during December 2016 to April 2017. Animals divided into seven experimental groups (n = 6) as follows: Group I served as a normal control (NC), maintained on regular rat pellet diet and drinking water ad libitum, and received distilled water as vehicle (5 ml/kg b.w., p.o.). Urolithiasis in remaining groups was induced by coadministration of 0.75% v/v EG and 1% w/v AC in drinking water ad libitum for first 3 days to augment lithiastic effect of EG, followed by 0.75% v/v EG alone for 18 days [15,16]. Group II served as disease control (DC) and receive distilled water (5 ml/kg b.w., p.o.). Group III and IV served as treatment groups and received MLG 200 and 400 mg/kg b.w., p.o., respectively, for 14 days (i.e., from day 8 to day 21). Group V and VI also served as treatment groups and received GC aqueous extract 100 and 200 mg/kg b.w., p.o., respectively, for 14 days. Group VII served as standard control and received Cystone® (CST) 400 mg/kg b.w., p.o. for 14 days. Drug interventions were initiated from day 8 onward till day 21 as illustrated in time scale of experimental protocol [Figure 1].

Collection and Biochemical Analysis of Urine

After 21 days of experimental period, rats were kept separately in metabolic cages. Urine samples over a period of 24 h were collected. Animals were given access to food and water ad libitum during urine collection. However, food was withdrawn 12 h before blood sample collection. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. Acidification of urine was done to prevent precipitation of calcium and magnesium by complexing with anions such as phosphate. Urinary excretion of calcium and phosphate was estimated using diagnostic kits as per manufacturer’s instructions while oxalate level was estimated as per Hodgkinson and Williams method [17].
Biochemical Analysis of Blood Serum

On day 22, blood samples were collected from overnight fasted animals by retro-orbital puncture under the influence of light ether anesthesia. Blood samples were subjected to centrifugation at 4000 rpm for 10 min and serum thus obtained was analyzed for calcium, phosphate, BUN, creatinine, and uric acid using respective kits.

Kidney Homogenate Preparation and Biochemical Analysis

After blood sample collection, animals were euthanized by cervical dislocation under the influence of sodium pentobarbital anesthesia (150 mg/kg b.w., i.p.). The kidneys were quickly excised and rinsed with ice-cold saline and blotted dry. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1 N hydrochloric acid for 30 min and homogenized. The kidney homogenate was centrifuged at 2000 rpm for 10 min. The supernatant was collected and used for the determination of calcium and phosphate levels using kits. For oxidative stress-related parameters, a portion of the kidney was minced to prepare a 10% w/v homogenate in Tris-HCl buffer (0.2 mol/L, pH 7.4) for the estimation of superoxide dismutase (SOD), catalase (CAT), and thiobarbituric acid-reactive substance (TBARS). SOD activity was determined by the method proposed by Marklund and Marklund [18] and was expressed as U/mg protein. CAT activity was assayed according to Aebi method [19] and expressed as nmol H₂O₂ consumed/min/mg protein. Formation of TBARS was measured by the modified method of Reddy and Lokesh [20] and presented as nmol TBARS/mg protein using nanomolar extinction coefficient of 1.56 × 10⁻⁵ cm⁻¹. Total protein in kidney homogenate was determined according to Lowery’s method as modified by Pomory [21].

Histopathology

A kidney from each group was stored in 10% buffered formalin after washing with cold normal saline for histopathological studies. The kidney was embedded in paraffin, and serial sections (3 µm thick) were cut using microtome. The sections were stained with hematoxylin and eosin (H and E) and were examined under light microscope and photographs were taken.

Statistical Analysis

The results were expressed as the mean ± standard error of mean (n = 6). Statistical analysis was performed by one-way ANOVA followed by Tukey-Kramer multiple comparison post hoc test using the GraphPad Prism version 6.01 for Windows, GraphPad Software, San Diego, CA, USA. P < 0.05 was considered statistically significant.

RESULTS

Effect of MLG and GC on Urine Parameters in EG- and AC-induced Urolithic Rats

Table 1 depicts the effect of MLG and GC on various urine parameters in EG- and AC-induced urolithiasis in rats. Lithogenic treatment significantly decreased urinary calcium excretion (P < 0.05) while increased phosphate (P < 0.05) and oxalate (P < 0.001) excretion in DC group animals when compared to that of NC group.

On the treatment with MLG 200, reduction in urinary elimination of urolithic promoters (phosphate and oxalate) remained non-significant (P > 0.05 for all parameters) while animals treated with MLG 400, GC 100, and GC 200 showed a significant decrease in urinary oxalate (P < 0.05 for MLG 400, P < 0.01 for GC 100, and P < 0.001 for GC 200) and increase urinary calcium excretion (P < 0.05 for MLG and P < 0.01 for GC 100 and 200) when compared to lithiatic rats. However, none of the treatment groups could decrease urine phosphate level (P > 0.05). Interestingly, the effect of GC 100 and GC 200 on urinary excretion of urilithic promoters was found to be similar, and no dose-dependent increase in the efficacy was observed.

Effect of MLG and GC on Serum Parameters in EG- and AC-induced Urolithic Rats

Table 1 summarizes the effect of MLG and GC on various serum parameters in EG- and AC-induced urolithiasis in rats. In the present study, a significant increase in serum concentration of BUN (P < 0.01), creatinine (P < 0.001), and uric acid (P < 0.001) was observed in lithiatic rats as compared to that of vehicle-treated animals. Except MLG 200-treated animals (P > 0.05 for all parameters), all other treatment groups showed a significant decrease in measured serum parameters. MLG 400 showed a significant decline in serum BUN (P < 0.05 for all) and creatinine (P < 0.01) while statistically non-significant reduction in uric acid (P > 0.05) was found when compared to DC group animals. On the other hand, animal groups treated with GC 100 (P < 0.01 for BUN, P < 0.001 for creatinine and P < 0.05 for uric acid) and GC 200 (P < 0.01 for BUN, P < 0.001 for creatinine, P < 0.05 for uric acid) produced significant reduction in all serum parameters when compared with positive control animals.
Effect of MLG and GC on Kidney Homogenate Parameters in EG and AC Lithiatic Rats

Table 2 depicts the effect of MLG and GC on various kidney homogenate parameters in EG- and AC-induced urolithiasis in rats. A significant elevation in lithiasis promoters (P < 0.01 for calcium, phosphate, and oxalate) was observed in the kidney of EG- and AC-administered rats when compared with NC animals. In MLG-treated groups, only MLG at a dose of 400 mg/kg b.w. could significantly reduce the levels of all three promoters of renal calculi (P < 0.01 for calcium and P < 0.05 for phosphate and oxalate), whereas MLG 200 did not show any beneficial effect in protecting the kidney (P > 0.05 for all calculi promoters). Animals treated with GC 100 and GC 200 showed significant decline in kidney homogenate calcium (P < 0.01 for both), phosphate (P < 0.01 for GC 100 and P < 0.001 for GC 200), and oxalate (P < 0.001 for both).

With respect to oxidative stress as depicted in Figure 2, administration of EG and AC significantly reduced antioxidant enzymes - SOD (P < 0.05) and CAT (P < 0.01) and elevated TBARS level (P < 0.001) in kidney homogenate of DC group animals when compared with that of NC group. Treatment with low and high doses of MLG (200 and 400 mg/kg b.w.) failed to restore SOD and CAT enzyme activities with only slight decrease in TBARS level (P > 0.05 for all) when compared with that of DC group animals. Contrary to that, GC in both doses (100 and 200 mg/kg b.w.) showed significant rise in kidney SOD (P < 0.05 for GC 100 and P < 0.001 for GC 200) and CAT levels (P < 0.01 for GC 100 and P < 0.001 for GC 200) when compared with that of DC group. Both doses of GC effectively replenished antioxidant enzymes to the normalcy in rat kidney. These results are further substantiated by histopathological examination of kidney sections of EG- and AC-administered rats [Figure 3]. With reference to GC treatment, both the doses (100 and 200 mg/kg b.w.) showed almost similar efficacy with GC 200 being slightly more potent than GC 100 in reducing promoters of urolithiasis in urine and kidney, as well as, restoring antioxidant enzyme levels in rat kidney.

DISCUSSION

A study has demonstrated that coadministration of EG (0.75% v/v) and AC (1% w/v) in drinking water to young male albino rats significantly accelerated magnitude of crystalluria. The same study further observed that EG causes 3-17 fold increase in urinary oxalate concentration while AC induces urine acidification and subsequent decrease of urinary citrate secretion which in turn leads to formation of CaOx crystals [22]. Deposition of CaOx crystals obstructs urine passage in renal tubules causing accumulation of nitrogenous waste products such as urea, creatinine, and uric acid in blood [23].

In the present study, serum BUN, creatinine, and uric acid were significantly increased due to EG- and AC-induced calculi formation in positive control animals when compared with vehicle-treated normal rats and are suggestive of renal damage. The observed abnormal changes in the serum parameters

Table 1: Effect of MLG and GC on urine and serum parameters in EG- and AC-induced urolithiatic in rats

| Groups | Treatment | Calcium (mg/g) | Phosphorous (mg/g) | Oxalate (mg/g) | BUN (mg/dL) | Creatinine (mg/dL) | Uric acid (mg/dL) |
|--------|-----------|----------------|--------------------|---------------|-----------|-------------------|-------------------|
| Groups I (NC) | Distilled water | 4.37±0.13 | 32.49±4.30 | 1.67±0.19 | 39.44±6.29 | 0.59±0.08 | 0.47±0.11 |
| Group II (DC) | Distilled water | 3.48±0.13 | 31.56±3.64 | 1.39±0.37 | 38.54±6.29 | 0.59±0.08 | 0.47±0.11 |
| Group III | MLG 200 mg/kg b.w. | 3.68±0.16 | 47.20±3.74 | 1.89±0.19 | 49.19±4.39 | 1.20±0.15 | 1.00±0.33 |
| Group IV | MLG 400 mg/kg b.w. | 4.34±0.19 | 45.48±3.13 | 2.09±0.08 | 43.33±3.23 | 0.99±0.16 | 0.76±0.29 |
| Group V | GC 100 mg/kg b.w. | 4.52±0.29 | 42.07±4.66 | 1.88±0.23 | 38.89±3.40 | 0.64±0.07 | 0.54±0.06 |
| Group VI | GC 200 mg/kg b.w. | 4.68±0.07 | 41.24±3.87 | 1.85±0.13 | 40.22±2.47 | 0.67±0.13 | 0.67±0.10 |
| Group VII | CST 400 mg/kg b.w. | 4.73±0.15 | 40.89±4.25 | 1.82±0.22 | 45.56±2.38 | 0.76±0.09 | 0.52±0.20 |

Values are expressed as mean±standard error of mean (n=6) and analyzed by one-way ANOVA followed by Tukey-Kramer multiple comparisons test. *P<0.05, **P<0.01, and ***P<0.001 when compared with NC group, *P<0.05, **P<0.01, and ***P<0.001 when compared with DC group. Not significant when compared with DC group, NC: Normal control, DC: Disease control, MLG: Magnesium lactate gluconate, GC: Garcinia cambogia, CST: Cystone®. EG: Ethylene glycol, AC: Ammonium chloride, BUN: Blood urea nitrogen

Table 2: Effect of MLG and GC on kidney homogenate parameters in urolithiatic rats

| Groups | Treatment | Calcium (mg/g) | Phosphorous (mg/g) | Oxalate (mg/g) |
|--------|-----------|----------------|--------------------|---------------|
| Groups I (NC) | Distilled water | 0.48±0.02 | 1.84±0.18 | 0.34±0.01 |
| Group II (DC) | Distilled water | 1.24±0.023 | 4.24±0.17 | 0.63±0.07 |
| Group III | MLG 200 mg/kg b.w. | 1.00±0.07 | 3.31±0.17 | 0.6±0.03 |
| Group IV | MLG 400 mg/kg b.w. | 0.76±0.17 | 2.72±0.57 | 0.46±0.04 |
| Group V | GC 100 mg/kg b.w. | 0.54±0.1 | 2.38±0.22 | 0.43±0.03 |
| Group VI | GC 200 mg/kg b.w. | 0.57±0.05 | 2.25±0.25 | 0.41±0.03 |
| Group VII | CST 400 mg/kg b.w. | 0.52±0.08 | 2.41±0.28 | 0.37±0.02 |

Values are expressed as mean±standard error of mean (n=6) and analyzed by one-way ANOVA followed by Tukey-Kramer multiple comparisons test. *P<0.01, and **P<0.001 when compared with NC group, *P<0.05, **P<0.01, and ***P<0.001 when compared with DC group. Not significant when compared with DC group, NC: Normal control, DC: Disease control, MLG: Magnesium lactate gluconate, GC: Garcinia cambogia, CST: Cystone®.
relevant to nephrolithiasis were in accordance with the reported studies [24,25]. Supplementation with MLG 400, GC 100, and GC 200 caused a marked reduction in serum BUN, creatinine, and uric acid.

A significant decrease in urinary calcium excretion and an increase in kidney homogenate calcium was observed in EG- and AC-administered rats. Similar observations have been reported by researchers [24,25]. Literature survey suggests that various acidic metabolites such as hippuric acid, oxalic acid, formic acid, and benzoic acids are formed on EG metabolism causing metabolic acidosis. Renal tubular acidosis eventually promotes renal calcium leakage which along with increased resorption of calcium from bones and increased intestinal absorption of calcium leads to hypercalciiuria and hypercalcemia [26-28].

Therefore, metabolic acidosis induced hypercalciiuria and hypocitraturia hastens nucleation and aggregation of CaOx crystals, potentiating the risk of calcium stone formation [29,30]. With respect to phosphate, it has been reported that increased urinary phosphate excretion couple with hyperoxaluria favors CaOx stone formation as CaP crystals act as nuclei for subsequent deposition of CaOx crystals [31]. Treatment groups showed increased elimination of calcium in urine and reduced urinary excretion of oxalate with no net effect on urinary phosphate level. Moreover, treatment with MLG and GC reduced all kidney homogenate parameters (calcium, phosphate, and oxalate) in EG- and AC-induced lithiatic rats when compared with DC group animals. However, there was not much difference in the efficacy of GC 100 and GC 200 which was comparable to that of reference standard CST 400. GC
200 was only slightly more potent than GC 100 in normalizing serum, urine, and kidney homogenate parameters. In other words, no dose-dependent benefits were observed with GC.

Hyperoxaluria is recognized as a far more significant risk factor in the pathogenesis of renal stones than hypercalciuria [32]. Co-administration of EG and AG in rats showed increased levels of phosphate and oxalate in urine and kidney homogenate, suggesting selective accumulation of these ions into renal parenchyma. Similar results have been observed in previously reported studies [24,25].

Multiple mechanisms have been proposed for hyperoxaluria in EG-induced lithiatic rats. Scientific studies have shown that EG is readily absorbed from the intestine and metabolized in the liver to oxalate causing hyperoxaluria [33,34]. Furthermore, increased availability of oxalate substrate in EG-administered animals activates oxalate-synthesizing enzymes (glycolic acid oxidase and lactate dehydrogenase), catalyzing oxidation, and reduction of glyoxylate and glycolate into oxalate leading to hyperoxaluria [35,36]. Excess oxalate present in urine forms water insoluble CaOx precipitates (by nucleation and aggregation) and subsequent retention, causing damage to epithelial linings of renal tubules [33,34].

The protective effect of MLG and GC could possibly be mediated through multiple mechanisms such as: (i) Ability to chelate Ca++ ion, thereby reducing availability for nucleation and aggregation of CaOx crystals in the renal tubules (hypocalciuria); (ii) reduction in oxalate in the urine and kidneys by interfering with EG metabolism and inhibiting oxalate-synthesizing enzymes (hyperoxaluria); and (iii) increasing rate of disintegration and dissolution of already formed CaOx stones in kidney tubules by forming water-soluble calcium complexes.

Magnesium has been documented for its stone formation inhibitory process [37]. It is postulated that MLG may react with CaOx and form metastable ion complexes with calcium to produce CLG which has significant water solubility than any other calcium salts (400 g/L) [11]. Formation of CLG might accelerate elimination of calcium into urine which in turn prevents CaOx crystal deposition in renal tubules. The reduction in abnormal levels of kidney stone promoters in respective biological samples is also suggestive of nephroprotection afforded by MLG and GC in lithiatic rats. The GC 200 and CST 400 treatments were equipotent in protecting rat kidneys from EG-induced damage.

The previous reports suggest that CaOx crystal deposition generates reactive oxygen species (ROS) in tubular epithelial cells. The cellular injuries favor the adherence and retention CaOx crystals in renal tubules [38]. In addition, researchers have demonstrated two-fold increase in xanthine oxidase in oxidative stress and subsequent renal damage in EG-administered rats [25]. A significant decrease in SOD and CAT activity in kidney homogenate was observed in lithiatic rats. Similar results have been reported previously [39-41].

In the present study, MLG 200 and MLG 400 were ineffective in restoring endogenous SOD and CAT enzymes in the kidney when compared with that of DC group. It is presumed that the failure of MLG in protecting kidneys from deleterious effects of ROS could be due to the fact that it does not possess any active phenolic groups in its structure. On the other hand, GC 100- and GC 200-treated groups showed restoration of SOD and CAT activity in the kidney and afforded dose-dependent protection of the kidneys from ROS-induced oxidative stress.

Another important biomarker of oxidative stress-induced lipid peroxidation is malondialdehyde which is a TBARS. A significant increase in kidney TBARS levels on EG and AC challenge is indicative of excessive ROS generation and subsequent depletion of endogenous antioxidant enzymes [39-41]. Accumulation of lipid peroxides may further damage EG-administered rat kidneys.

In the present investigation, animals groups treated with MLG 200 and MLG 400 did not reduce TBARS levels in the kidneys which further corroborate its lack of antioxidant property. Contrary to this, GC 100- and GC 200-treated rats showed a significant decline in TBARS levels in kidney homogenate of lithiatic rats.

Antioxidants such as Vitamin C, catechin, and selenium have shown protection against CaOx crystal deposition induced oxidative injury [42,45]. Therefore, the nephroprotective effect of GC could also be attributed to its antioxidant phenolic compounds, such as gallic acid and flavonoids [44]. A previous study has noted that citrate and hydroxycitrate are equally effective as inhibitors of CaOx monohydrate (COM) nucleation. However, both molecular inhibitors of COM crystallization exhibit different mechanisms. Hydroxycitrate acts by adsorption on crystal surfaces, thereby inducing dissolution of the crystal rather than a reduced rate of crystal growth. The study further reports that hydroxycitrate makes the surface of CaOx crystals smoother to prevent subsequent crystal deposition [45]. In the present investigation, superior nephroprotection afforded by GC can further be substantiated by histopathological studies. Microscopic examination of H and E stained kidney sections revealed that there was a significant dilatation of renal tubules with deposition of CaOx crystals in DG group animals. Furthermore, the loss of granularity and detachment of tubular epithelial cells suggestive of renal damage. Thus, ROS generation coupled with deposition of CaOx crystals has caused significant damage to the renal tubular cells. Treatment with GC extract showed significant protection against oxidative stress-induced cellular damage in kidneys, and this could be attributed to its phenolic phytochemicals.

**CONCLUSION**

The nephroprotection afforded by MLG was limited to its inhibitory effect on CaOx crystal deposition in nephron tubules as it lacked antioxidant effects. On the other hand, GC 100- and 200-treated rats showed superior protection of the kidneys with in terms of reduction in the levels of kidney stone promoters in serum/urine, decrease in CaOx crystals deposition in renal tubules, and preservation of cellular architecture of nephrons.
is, therefore, contended that MLG and GC could be considered as potential alternatives for safer and effective management of CaOx-induced urinary stones.

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