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Clinical-grade *Garcinia cambogia extract* dissolves calcium oxalate crystals in *Drosophila* kidney stone models

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Abstract. – OBJECTIVE: Kidney stone formers have a high rate of stone recurrence after kidney stone removal surgery and there is no effective medication for treatment. Hydroxycitric acid (HCA), which is the major component of Garcinia cambogia extract, can dissolve calcium oxalate crystals in vitro, suggesting that Garcinia cambogia could be used to treat calcium oxalate kidney stone. In this study, we used the Drosophila kidney disease model to evaluate the effect of Garcinia cambogia on the prevention and removal of calcium oxalate stones *in vivo*.

MATERIALS AND METHODS: Flies were reared in fly food containing different concentrations of GCE for one week. The effect of GCE on preventing the formation of calcium oxalate stone was examined. WT and v-ATPase gene RNAi knockdown flies were reared in fly food with 0.3% NaOx for one week, then fed different concentrations of GCE for one week. The effect of GCE on the removal of calcium oxalate stone was examined.

RESULTS: Garcinia cambogia extract dissolves calcium oxalate crystals from Malpighian tubules in both genetic and non-genetic Drosophila kidney stone models compared to citric acid. Hydroxycitric acid also directly dissolves calcium oxalate crystals in Drosophila Malpighian tubules ex vivo.

CONCLUSIONS: Garcinia cambogia extract removes calcium oxalate kidney stones from Drosophila Malpighian tubules via directly dissolving calcium oxalate stones by HCA. Our study strongly suggests that clinical-grade Garcinia cambogia extract could be used to treat patients with nephrolithiasis in the future.

Key Words:
Garcinia cambogia extract, Hydroxycitrate, Calcium oxalate kidney stone, Nephrolithiasis, Drosophila genetic model, Malpighian tubule.

Introduction

The incidence of nephrolithiasis is increasing globally and gradually puts a huge burden on health care system. Calcium-containing kidney stones are the most common type of kidney stone and account for more than 90% of renal stone diseases[1]. Approximately half of calcium oxalate (CaOx) stone formers are idiopathic[2,3], kidney stones have been thought to be the result of the interaction between genetic and environmental factors, including dehydration stemming from low fluid intake and high dietary intake of animal protein and salts. Meanwhile, more than 30 genes have been identified as novel monogenic causes of kidney stone disease using whole exome sequencing[1,4-8]. There are currently no effective medications and the most advanced treatment for kidney stones is minimally invasive kidney stone surgical procedures, such as ureteroscopy and laser lithotripsy, shockwave lithotripsy (SWL), and percutaneous nephrolithotomy (PCNL) depending on the size and type of stones. Great improvement in surgical techniques used to remove kidney stones have been made in the last two decades, patients often experience recurrence after surgery. The surgical burden and recurrence associated with these patients demand new drugs that dissolve kidney stones *in situ*. However, no major progress has been made to remove kidney stone formation in the last three decades, because of the lack of ideal animal models for high-throughput drug screening.

The *Drosophila* excretory system is composed of Malpighian tubules and nephrocytes[9]. It has been shown that the *Drosophila* nephrocyte shares remarkable similarity with the glomerular podocyte for protein ultrafiltration. There is also remarkable similarity between nephrocyte and the renal proximal tubule for protein reabsorption[9-12]. The *Drosophila* Malpighian tubule shares striking structural and functional similar features with mammalian renal tubules and collecting ducts[13].

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The principal cells and stellate cells are two types of cells in *Drosophila* Malpighian tubule, which contain many organic solute transporters[14]. The principal cells are the major tubular cell type (~80%) through which cations and organic solutes are transported. The stellate cells are the minor tubular cell type (~20%) through which chloride ion and water flow and interspersed at regular intervals with the principal cells. Urine is generated through active transport of ions, water and organic solutes from the hemolymph into the Malpighian tubule lumen[13]. *Drosophila* Malpighian tubule is a powerful model system to investigate the pathogenesis of human nephrolithiasis[14-19]. In Malpighian tubule, calcium oxalate stones are formed with the addition of lithogenic agents in fly food and can be directly examined under polarized light microscopy[20]. Slc26a6 regulates oxalate secretion in human renal tube functioning as a $Cl^-/HCO3^-$ exchanger. Mutations of Slc26a6 were identified from patients with nephrolithiasis[21]. RNAi knockdown of dPrestin (the *Drosophila* homolog of Slc26a6) led to decreased calcium oxalate stone formation in Malpighian tubules[22]. Therefore, fruit fly is an ideal genetic kidney stone disease model to screen new genes involved in the pathogenesis of nephrolithiasis and validate the function of candidate genes identified from patients with nephrolithiasis *in vivo*[17].

Drosophila kidney calcium oxalate stone models have been used to screen traditional Chinese medicinal herbs for nephrolithiasis treatment[23]. Wu et al[16] showed that Traditional Chinese Medicine herbs have a potential antilithic effect, but it is still unclear whether they can directly dissolve kidney stones *in situ*. *Garcinia cambogia* has been widely used for cooking in Southern Asia. *Garcinia cambogia* extract has been used as a supplement for weight-loss in USA for over 40 years. A recent study showed that HCA induces the dissolution of the calcium oxalate crystal *in vitro* and *Garcinia cambogia* could be used to treat calcium oxalate kidney stone[24]. In this study, we used the *Drosophila* genetic and non-genetic kidney stone model to examine whether *Garcinia cambogia* and hydroxycitrate dissolve calcium oxalate crystals *in vivo*. Our result showed that *Garcinia cambogia* extract can prevent calcium oxalate crystals formation and remove calcium oxalate crystals in Malpighian tubules *in vivo*. We also showed that hydroxycitrate can directly dissolve calcium oxalate crystals in Malpighian tubules *ex vivo*. Our result suggest that *Garcinia cambogia* extract might be used to treat calcium oxalate kidney stones in clinic.

Materials and Methods

Fly Strains

All flies were reared on standard food. UAS-Gal4 crosses were performed at 25°C. Uro-Gal4 (Bl-44416)[25] and UAS-nGFP (Bl-4775) were obtained from the Bloomington *Drosophila* stock center. UAS-RNAi transgenic fly lines targeting vha100-2 (TH04790.N, Bl-64859) and vha55 (THU4117 and v-46554), referred in the main text and figures as vha100-2 IR and vha55 IR, were obtained from the Bloomington *Drosophila* stock center, Vienna stock center and Tsinghua Fly center. Uro-Gal4 and UAS-nGFP were recombined together to label principal cells at all developmental stages.

Chemicals

Potassium hydroxycitrate tribasic monohydrate (59847) and Potassium citrate tribasic monohydrate (c8385) were purchased from Sigma-Adlrich (St. Louis, MO, USA). Swanson® Super Citrimax Clinical Strength AND Swisse® Healthy Plan *Garcinia cambogia extact* were purchased online from Amazon (Tables I and II). Other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise indicated.

RNAi Knockdown of Nephrolithiasis-Related Genes in Principal Cells

This method was adopted from our recent study described previously[11]. Briefly, the UAS/Gal4 system allows for the over-expression or knockdown of "gene-of-interest" in a cell-specific manner in *Drosophila*. A fly line that possesses a "GAL4-driver" can be crossed to a second transgenic fly line containing a construct of interest gene X placed downstream of a UAS promoter sequence. This allows the downstream transgene to be expressed specifically in these cells where GAL4 is expressed. To specifically knock down

Table I. Supplement Facts of Swanson® Super Citrimax Clinical Strength capsules (Serving size 2 capsules).

Component	Amount/serving
Garcinia cambogia extact	1.5 g/serving
Hydroxycitric acid (60%)	900 mg/serving
Calcium	120 mg/serving
Potassium	180 mg/serving
Gelatin	N. A.
Magnesium stearate	N. A.
Stearic acid	N. A.
Microcrystalline cellulose	N. A.

nephrolithiasis- related genes in Malpighian tubule principal cells, we used the Uro-GAL4 driver, which drives gene expression specifically in the tubular principal cells, and crossed it to the UAS-RNAi lines containing a dsRNA hairpin directed against gene X.

RNAi-Based Functional Analysis of Malpighian Tubule Genes

10 virgins of Uro-Gal4/UAS-nGFP flies were crossed with 5 males of UAS-RNAi transgenic line in vials at 25°C. Freshly hatched flies were transferred to fly food with 0.3% NaOx at 25°C for 1 week. Malpighian tubules were dissected in phosphate-buffered saline (PBS) under dissection microscope and then subjected to the examination of renal stone in Malpighian tubules under polarized white light with an Olympus (Tokyo, Japan) BX63 optical microscope. We took images for the whole four tubules, and then we randomly selected three images for quantification. Renal stone formation was measured in the whole field of view (700 μm × 100 μm, 20× magnification) using CellSens software. The results were expressed as mean ± SD (standard deviation) (n=10). All statistical analyses were performed using GraphPad Prism5 software (La Jolla, CA, USA). Statistical significance was defined as $p<0.05$.

Calcium Oxalate Crystal Prevention Analysis In Vivo

Wild type or mutant flies were reared on regular fly food with 0.1% NaOx and different concentration of HCA or GCE for one week. Malpighian tubules of adult female flies were dissected in PBS under dissection microscope, then subjected to the examination of renal crystals in Malpighian tubules under polarized white light with an Olympus (Tokyo, Japan) BX63 optical microscope. We took images for the whole four tubules, and then we randomly selected three images for quantification. Renal stone formation was measured in the whole field of view (700 μm × 100 μm, 20× magnification) using cellSens software. The results were expressed as mean ± SD (n=10). All statistical analyses were performed using GraphPad Prism5 software (La Jolla, CA, USA). Statistical significance was defined as $p<0.05$.

Calcium Oxalate Crystals Removal Assay In Vivo

First, wild type or mutant flies were reared on regular fly food containing 0.3% NaOx at 25°C for one week and renal tubule crystals formation was evaluated briefly. Then, flies were transferred to fly food containing 0.1% NaOx and different concentration of HCA or GCE at 25°C for 1 week. Malpighian tubules of adult female flies were dissected in PBS under dissection microscope and then subjected to the examination of renal crystals in Malpighian tubules under polarized white light with an Olympus (Tokyo, Japan) BX63 optical microscope. We took images for the whole four tubules, and then we randomly selected three images for quantification. Renal crystal formation was measured in the whole field of view (700 μm × 100 μm, 20× magnification) using cellSens software. The results were expressed as mean ± SD (n=10). All statistical analyses were performed using GraphPad Prism5 software (La Jolla, CA, USA). Statistical significance was defined as $p<0.05$.

Ex Vivo Calcium Oxalate Crystal Dissolution Analysis

Briefly, wild type w^{1118} flies were reared on regular fly food containing 0.3% NaOx for one week. Intact Malpighian tubules were dissected in PBS under dissection microscope and transferred onto a slide. 100 μL CA, HCA or GCE solution was added to completely cover the MT tissue, and then the Malpighian tubules were subjected to live-imaging under polarized white light with an Olympus (Tokyo, Japan) BX63 optical microscope without a coverslip. Images were taken every 20 min and the total area of renal crystals was measured using cellSens software. Renal crystal dissolution rate was calculated by dividing the remaining crystal area by the total crystal area at the beginning. The results were expressed as mean ± SD (n=5). All statistical analyses were performed using GraphPad Prism5 software (La Jolla, CA, USA).

Statistical Analysis

Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) 22. 0

Table II. Ingredients of Swisse® Healthy Plan Garcinia cambogia extact.

Component	Amount/serving
Garcinia cambogia extact (containing 60% Hydroxycitric acid)	35%
Calcium phosphate	N. A.
Hypromellose	N. A.
Maltodextrin	N. A.
Magnesium stearate	N. A.
Silicon dioxide	N. A.

software (IBM Corp., Armonk, NY, USA). Data were represented as mean ± Standard Deviation (SD). The *t*-test was used for analyzing measurement data. Differences between two groups were analyzed by using the Student's *t*-test. Comparison between multiple groups was done using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). $p<0.05$ indicated the significant difference.

Results

Clinical-grade Garcinia Cambogia Extract Prevents Calcium Oxalate Kidney Crystal Formation in Drosophila Renal Tubules In Vivo

To examine whether *Garcinia cambogia* can prevent calcium oxalate crystals formation *in vivo*, w^{1118} wild type flies were reared in fly food with 0.1% NaOx and different concentrations of GCE for one week, and then the effect of GCE on kidney crystal formation in renal tubules was examined. High concentration of NaOx affects the survival of flies (Figure 1), so we used 0.1% and 0.3% NaOx in our following experiments. *Garcinia cambogia* extract prevented calcium oxalate kidney crystal formation in adult renal tubules (Figure 2A-2D). Compared to hydroxycitrate or citrate, *Garcinia cambogia* extract prevented calcium oxalate crystal formation in *Drosophila* renal tubules at a low concentration (Figure 2E). On the other hand, citric acid or HCA partially blocked calcium oxalate crystal formation in *Drosophila* renal tubules at a high concentration (Figure 2E). Both of them prevented calcium oxalate renal crystal formation in *Drosophila* renal tubules at a very high concentration (Figure 2E). These results suggest that *Garcinia cambogia* extract is a better reagent to prevent calcium oxalate crystal formation in kidney stone disease models *in vivo* than citric acid.

Garcinia Cambogia Extract Removes Renal Calcium Oxalate Crystals from Drosophila Renal Tubule In Vivo

Hydroxycitric acid dissolves calcium oxalate crystals *in vitro*. We reasoned that *Garcinia cambogia* extract containing 60% HCA plays a sim-

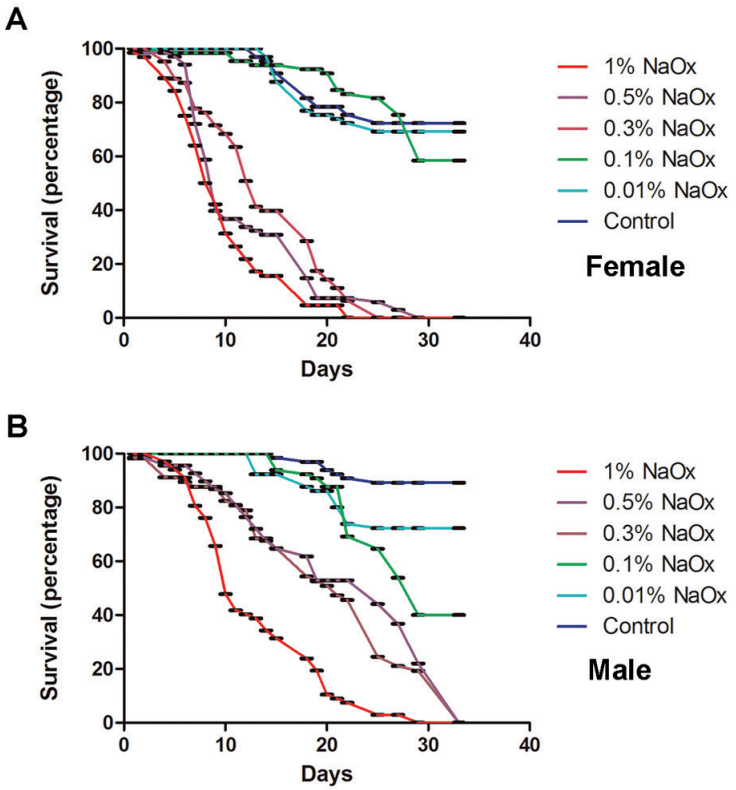

Figure 1. Effect of Sodium Oxalate (NaOx) on the survival of Drosophila adult. Survival rate of female (**A**) and male (**B**) w^{1118} wild type flies reared on regular fly food containing different concentration of NaOx (0, 0.01%, 0.1%, 0.3%, 0.5% and 1%) at 25°C.

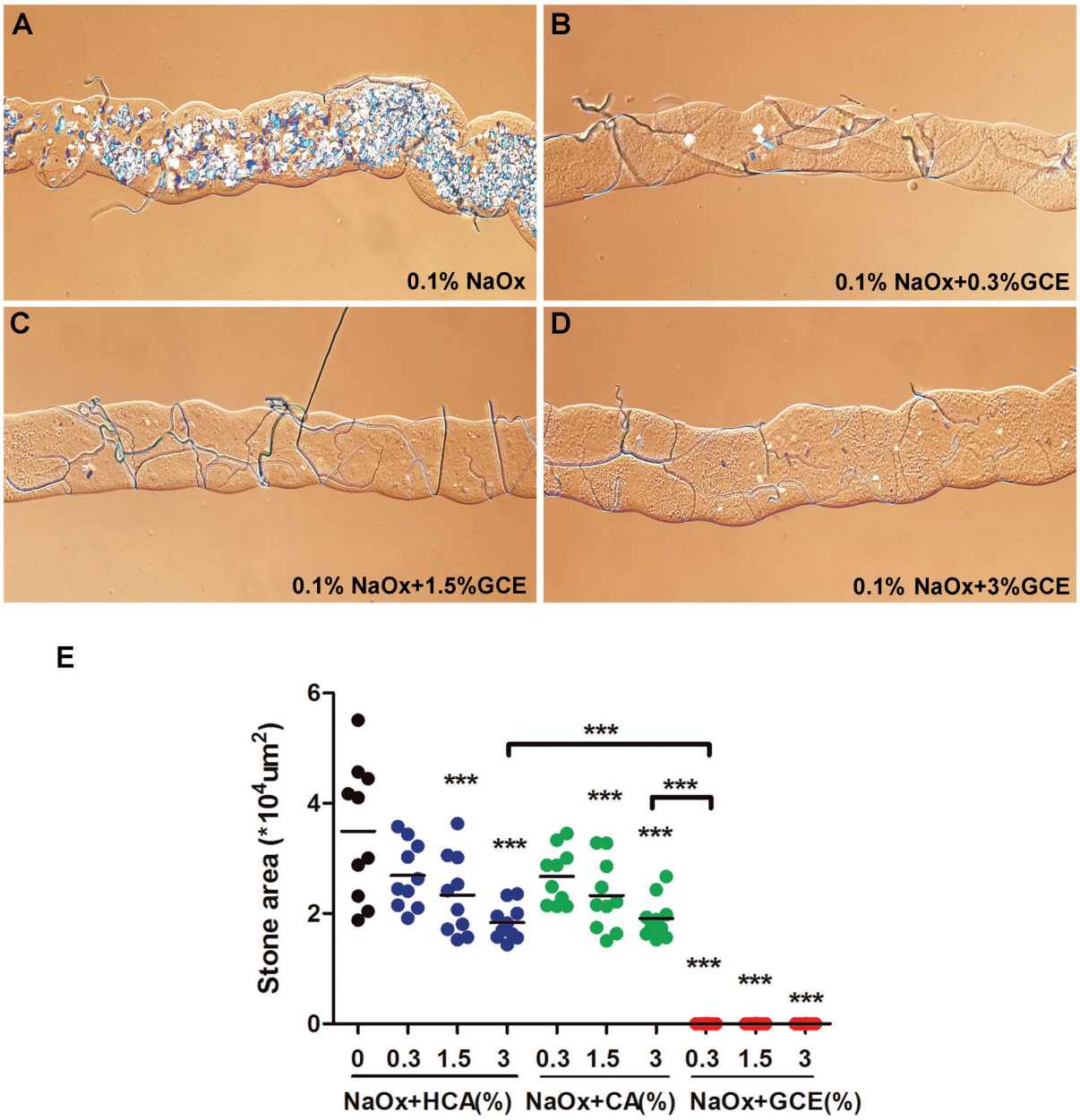

Figure 2. *Garcinia cambogia* extract prevents calcium oxalate crystal formation in *Drosophila* renal tubules. Representative images of the effect of GCE on the formation of CaOx crystal in adult malpighian tubules (**A-D**). Calcium oxalate kidney crystal formation in wild type flies reared in fly food containing 0.1% NaOx (**A**), 0.1% NaOx+0.3% GCE (**B**), 0.1% NaOx+1.5% GCE (**C**), 0.1% NaOx+3% GCE (**D**). 10 pairs of Malpighian tubules from 5 flies per genotype were dissected and analyzed. Total area of CaOx crystals in Malpighian tubule was measured in the whole field of view (700 µm × 100 µm, 20× magnification). The results are expressed as mean ± SD. One-way ANOVA was performed to analyze the data and Bonferroni's multiple comparison was performed to compare all pairs of columns. Statistical significance was defined as $p<0.05$ (*$p<0.05$, **$p<0.005$, ***$p<0.0005$). **E,** Comparison of the effect of GCE, HCA and CA on calcium oxalate crystal formation. GCE totally prevented calcium oxalate crystal formation in adult renal tubules at 0.3%, 1.5% and 3% compared to control (***$p<0.0001$); whereas, 3% of HCA or CA only partially prevented calcium oxalate kidney stone formation compared to control (***$p<0.0001$). 0.3% of GCE is more efficient to prevent calcium oxalate kidney stone formation compared to 3% of HCA or CA (***$p<0.0001$).

ilar role *in vivo*. To investigate the effect of GCE on calcium oxalate crystals removal *in vivo*, we developed a calcium oxalate renal stone model. Flies were fed fly food containing 0.3% NaOx for one week. Then, flies were transferred to fly food which contains 0.1%NaOx and different concentrations of GCE for one week. The effect of GCE on kidney crystal removal was examined.

As shown in Figure 3, *Garcinia cambogia* extract removed calcium oxalate renal crystals from renal tubule in a concentration-dependent manner (Figure 3A-3D). The total crystal area was reduced 93% in renal tubules of flies reared in fly food containing 0.3% GCE compared to flies reared in 0.1% NaOx. There are no crystals left in renal tubules of flies reared in fly food with 1.5% (Figure 3E) or 3% *Garcinia cambogia* extract (Figure 3E). Compared to *Garcinia cambogia* extract,

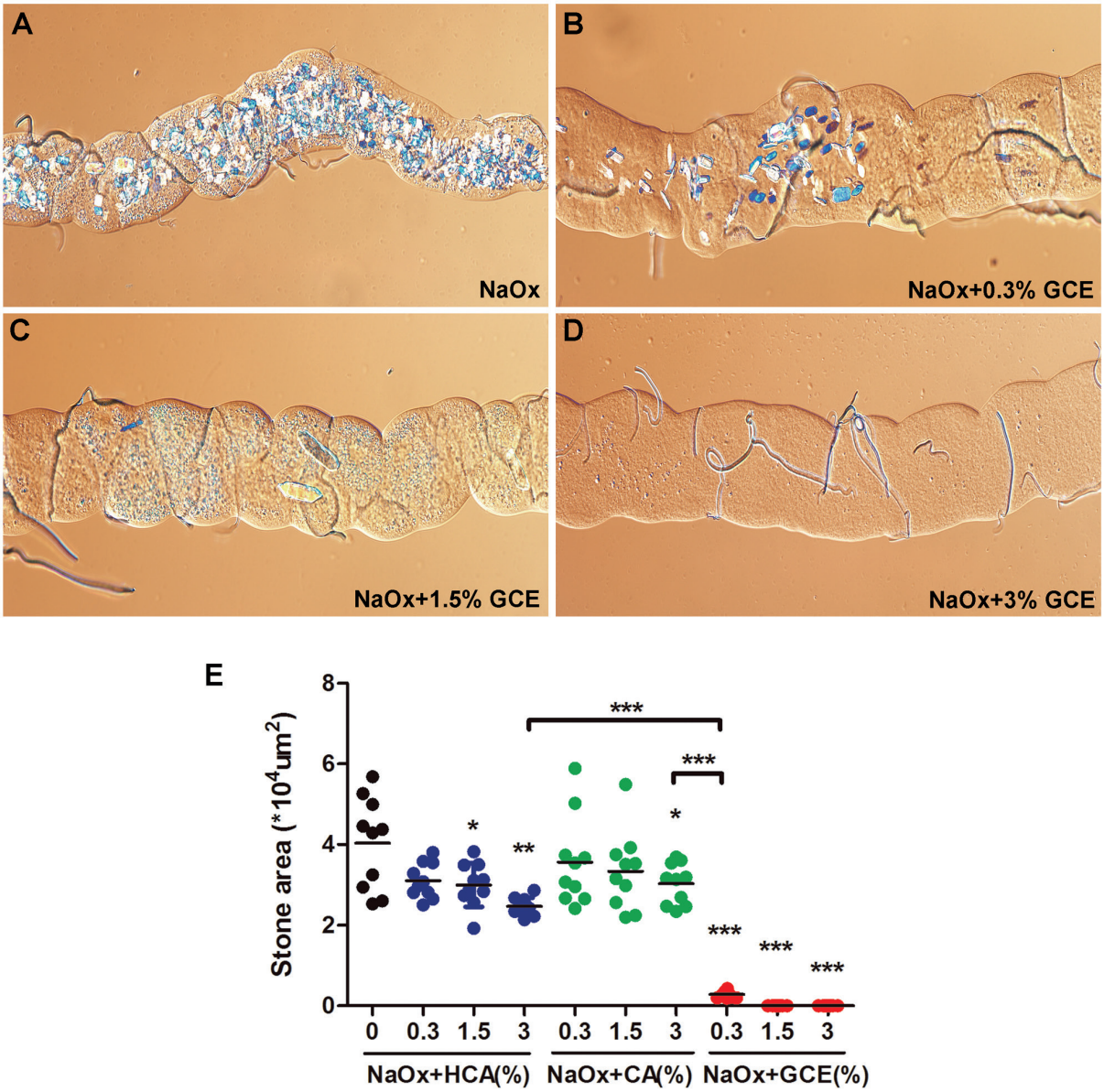

Figure 3. *Garcinia cambogia* extract completely removes calcium oxalate crystal from *Drosophila* renal tubules *in vivo*. Representative images of the effect of GCE on the removal of CaOx crystals in adult Malpighian tubules (**A-D**). Wild type flies were reared in fly food containing 0.3% NaOx for one week and then transferred to new fly food containing 0.1% NaOx (**A**), 0.1% NaOx+0.1% GCE (**B**), 0.1% NaOx+0.5% GCE (**C**), 0.1% NaOx+1% GCE (**D**) for one week. 10 pairs of Malpighian tubules from 5 flies per genotype were dissected and analyzed. Total area of CaOx crystals in Malpighian tubule was measured in the whole field of view (700 µm × 100 µm, 20× magnification). The results are expressed as mean ± SD. One-way ANOVA was performed to analyze the data and Bonferroni's multiple comparison was performed to compare all pairs of columns. Statistical significance was defined as $p<0.05$ (*$p<0.05$, **$p<0.005$, ***$p<0.0005$). **E,** Comparison of the effect of GCE, HCA and CA on calcium oxalate crystal removal. Almost all calcium oxalate crystals in adult renal tubules were removed in the presence of 1.5% or 3% GCE compared to control (***$p<0.0001$), whereas, only 40% of calcium oxalate crystals were removed even in the presence of 3% HCA or CA(**$p<0.005$ or *$p<0.05$). 0.3% of GCE is more efficient to remove calcium oxalate crystal formation compared to 3% of HCA or CA (***$p<0.0001$).

0.3% and 1.5% hydroxycitric acid removed calcium oxalate kidney crystals less efficiently (Figure 3E). Compared to flies reared in 0.1% NaOx group, total crystal area was reduced by 50% in renal tubules of flies reared in fly food with 0.3% HCA. 3% of citric acid partially removed calcium oxalate crystals in *Drosophila* renal tubules. Compared to the flies in control group, the total crystal area was reduced by 20% (Figure 3E).

Garcinia Cambogia Extract Has a Similar effect in Genetic Calcium Oxalate Kidney Stone Drosophila Model

To examine whether *Garcinia cambogia* extract has a similar effect in genetic calcium oxalate renal stone model, we used UAS/Gal4 system to generate a genetic calcium oxalate kidney stone *Drosophila* model[26]. Mutations in ATP6V1B1 and ATP6V0A4 have been identified in calcium oxalate kidney stone patients, suggesting that they are essential calcium oxalate kidney stones formation[27]. Vha55 and Vha100-2, fly homologs of ATP6V1B1 and ATP6V0A4, are highly expressed in Malpighian tubules. To specifically silence Vha55 and Vha100-2 genes in Malpighian tubule principal cells, we used the Uro-GAL4 that specifically drives gene expression in renal principal cells[13,25,26,28,29] (Figure 4), and crossed it to the UAS-RNAi lines containing a dsRNA hairpin directed against Vha55 and Vha100-2. RNAi knockdown of V-ATPase subunits resulted in increased calcium oxalate crystal formation (Figure 5). Vha55 and vha100-2 RNAi knockdown resulted in increased calcium oxalate crystal formation in Malpighian tubules compared to control group (Figure 6A, 6D, 6G). *Garcinia cambogia* extract removed calcium oxalate crystals from renal tubules of vha55/vha100-2 RNAi knockdown flies in a concentration-dependent manner (Figure 6A-6J). Compared to flies reared in 0.1% NaOx only, total crystal area was reduced to 12.68% in (0.1% NaOx+0.5% GCE) group, and 0% in (0.1% NaOx+1% GCE) group. Our results showed that *Garcinia cambogia* extract removed calcium oxalate crystal in genetic calcium oxalate kidney stone *Drosophila* model.

Hydroxycitric Acid Dissolves Calcium Oxalate Crystals in Drosophila Renal Tubules Ex Vivo

To investigate the molecular mechanism through which *Garcinia cambogia* extract removes calcium oxalate crystals from renal tubules, we examined the dissolution of calcium oxalate crystals in the Malpighian tubule. Hydroxycitric acid dissolved calcium oxalate crystals in renal

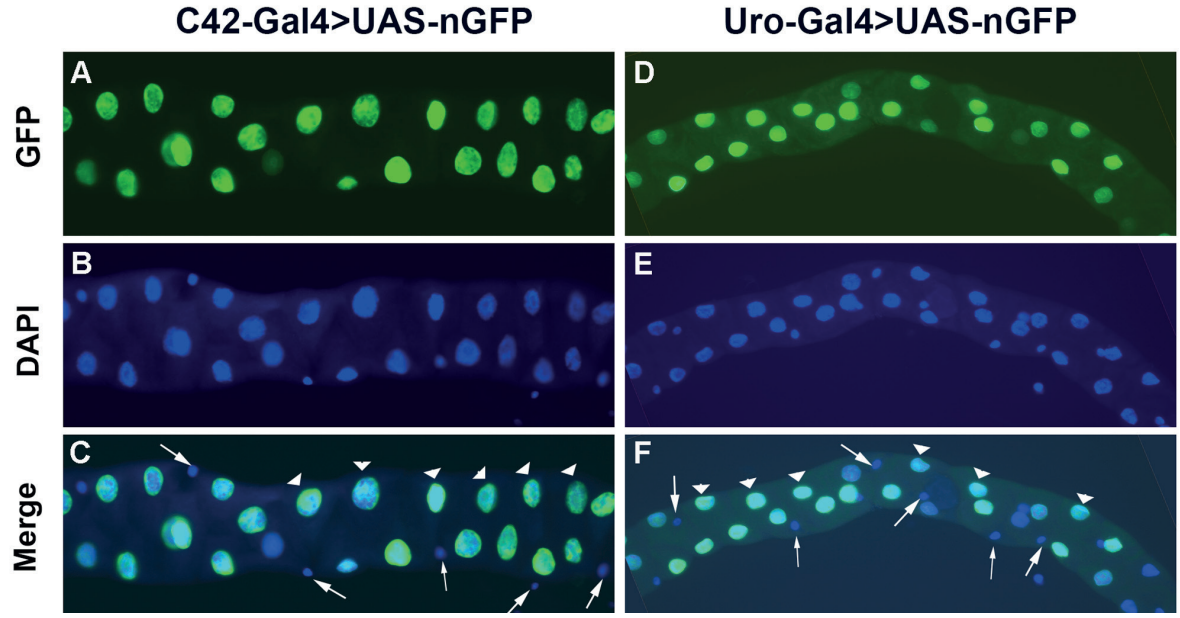

Figure 4. Uro-Gal4 and C42-Gal4 are specifically expressed in principal cells of *Drosophila* malpighian tubule. Both C42-Gal4 (**A-C**)**,** and uro-Gal4 (**D-F**,) are specifically expressed in principal cells of *Drosophila* malpighian tubules. nGFP was co-localized with DAPI in principal cells (larger nucleus), but not in stellate cells (small nucleus). Arrowheads depicted principal cells and arrows depicted stellate cells.

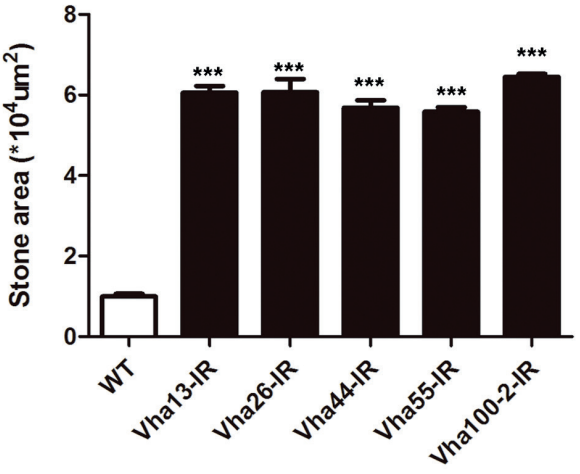

Figure 5. The effects of fly orthologs of 6 mammalian v-ATPase genes on CaOx crystal formation in Malpighian tubules. RNAi Knock-down of each gene of v-ATPase complex led to increased formation of calcium oxalate crystal in *Drosophila* Malpighian tubule. The results are expressed as mean ± SD. The unpaired T-test was performed with two-tailed *p*-values and 95% confidence intervals. Statistical significance was defined as $p<0.05$. (*$p<0.05$, **$p<0.005$, ***$p<0.0005$).

tubules *ex vivo* in a concentration- and time- dependent manner (Figure 7A-F). No crystals were excreted from the Malpighian tubule during our observation. 50% CaOx crystals were dissolved in 0.5% HCA solution and were completely dissolved in 1% HCA and 1% CA within 2 hours. *Garcinia cambogia* extract has no effect on the dissolution of calcium oxalate stones pre-formed in the Malpighian tubule (Figure 7G).

Discussion

No major progress has been made in drug discovery for kidney stone treatment due to the lack of ideal animal models which can be used for high-throughput drug screen in the past 30 years. *Drosophila* Malpighian tubule recently has become a model system to study the pathogenesis of human nephrolithiasis. In this study, using UAS-RNAi/Uro-Gal4 system, we found that RNAi knock-down of V-ATPase genes led to calcium oxalate crystal formation in *Drosophila* Malpighian tubules, suggesting that V-ATPase complex in principal cells is critical to prevent calcium oxalate stone formation.

Garcinia cambogia extract contains 60% hydroxycitric acid and is a weight-loss supplement which has been used for more than 40 years. Recently, it has been shown that hydroxycitric acid can dissolve calcium oxalate crystals *in vitro*, suggesting that *Garcinia cambogia* extract might be used to treat calcium oxalate kidney stones[24]. The novelty of this study was that *Garcinia cambogia* extract directly dissolves calcium oxalate crystals *in vivo*. In this study, our data showed that *Garcinia cambogia* extract prevented calcium oxalate crystal formation in *Drosophila* renal tubules. We also showed that *Garcinia cambogia* extract removed calcium oxalate crystals from *Drosophila* renal tubules in a concentration-dependent manner. On the other hand, hydroxycitric acid had no effect on calcium oxalate renal crystal formation and removal in *Drosophila* Malpighian tubules. We speculated that hydroxycitric acid added in fly food could not be absorbed and transported to Malpighian tubules due to the lack of key components in *Garcinia cambogia* extract, which were dispensable for hydroxycitric acid delivery to Malpighian tubules. Our data showed that *Garcinia cambogia* extract was effective in both genetic and non-genetic *Drosophila* kidney stone models, suggesting that *Garcinia cambogia* extract might be a new drug for kidney stone disease.

Our data also demonstrated that hydroxycitric acid directly dissolves calcium oxalate crystals in *Drosophila* renal tubules *ex vivo*. Calcium oxalate crystals in dissected renal tubules were completely dissolved in 0.5% and 1% hydroxycitric acid. However, 2% *Garcinia cambogia* extract which contains 60% hydroxycitric acid had no effect on the dissolution of calcium oxalate crystals in Malpighian tubules *ex vivo*. We tried to examine the presence of hydroxycitric acid in the water solution using LC-mass spectrometry, we did not detect hydroxycitric acid in 1% *Garcinia cambogia* extact water solution. We reasoned that this was because 31.3% of *Garcinia cambogia* extact is composed of fiber, which cannot be dissolved in water, and hydroxycitric acid could not be released from *Garcinia cambogia* extact water solution. We actually used ethanol solution to perform similar experiments, there is no effect on the dissolution of calcium oxalate crystals *ex vivo*. It is not clear how hydroxycitric acid is absorbed and released *in vivo*. The fact that we did not observe any impact of *Garcinia cambogia* extact on the removal of calcium oxalate stones *ex vivo*, suggest that the release of hydroxycitric acid from *Garcinia cambogia* extact is essential for its function to remove the calcium oxalate crystals *in vivo*. Hydroxycitric acid might be absorbed in the midgut and released into hemolymph, and then

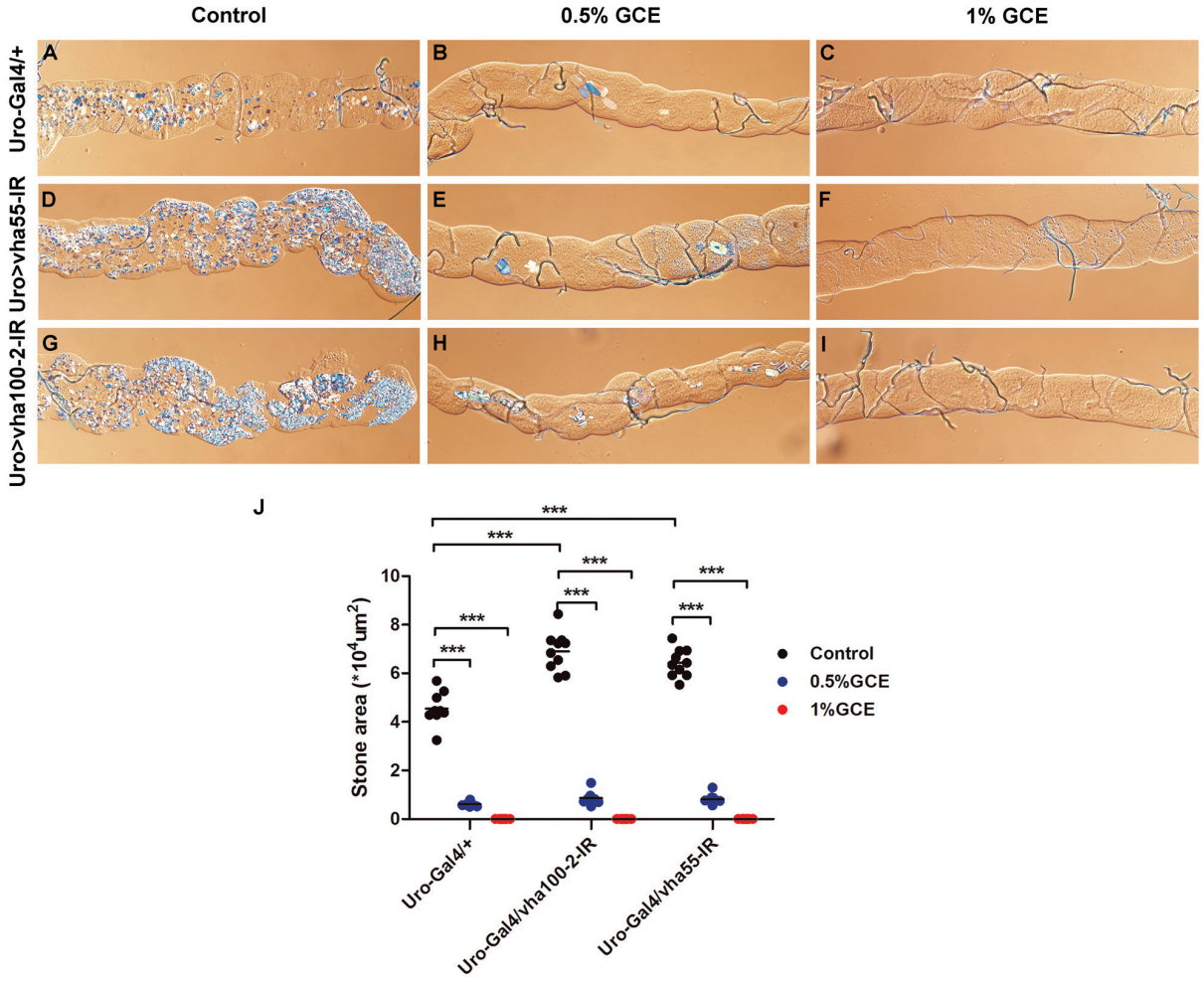

Figure 6. *Garcinia cambogia* extract efficiently removes calcium oxalate crystals in genetic nephrolithiasis *Drosophila* model. Representative images of the effect of GCE on the removal of CaOx crystal in adult Malpighian tubules of control Uro-Gal4/+ (**A-C**), Uro-Gal4/vha55 RNAi knockdown (**D-F**) and Uro-Gal4/vha100-2 RNAi knockdown flies (**G-I**). Flies reared in fly food containing 0.3% NaOx for one week were transferred to food containing 0.1% NaOx, 0.1% NaOx+0.5% GCE, 0.1% NaOx+1% GCE for one week. 10 pairs of Malpighian tubules from 5 flies per genotype were dissected and analyzed. Total area of CaOx crystals in Malpighian tubule was measured in the whole field of view (700 μm × 100 μm, 20× magnification). The results are expressed as mean ± SD. Two-way ANOVA grouped analyses were performed to analyze the data and Bonferroni's post-test was performed to compare replicate means by row. Statistical significance was defined as $p<0.05$ (*$p<0.05$, **$p<0.005$, ***$p<0.0005$). **J,** Comparison of the effect of GCE on the removal of calcium oxalate crystals in adult Malpighian tubules of control, vha55 RNAi knockdown and vha100-2 RNAi knockdown flies. RNAi knock-down of vha55 and vha100-2 led to increased formation of calcium oxalate crystals in Malpighian tubules compared to control (Uro-Gal4/+) (***$p<0.0001$). All calcium oxalate crystals in adult renal tubules were removed in the presence of 1% GCE in all three groups.

secreted into Malpighian tubule where hydroxycitric acid could dissolve calcium oxalate crystals. Our results suggest that *Garcinia cambogia* extract removes calcium oxalate renal crystals from Malpighian tubule by direct dissolution of calcium oxalate crystals by hydroxycitric acid.

Garcinia cambogia extract has been widely used as a supplement for weight loss for over 40 years in USA. The results from most of studies on the toxicity of *Garcinia cambogia* extract in clinical trials indicate that *Garcinia cambogia* extract is safe to use.

There are some limitations in our current study. The absorption and metabolism of hydroxycitric acid might be different between *Drosophila* and mammals. The size of the stones in human and *Drosophila* are different. New finding from *Drosophila* disease model cannot directly be applied to mammalian system. Currently, we are investigating the effect of *Garcinia cambogia* extract

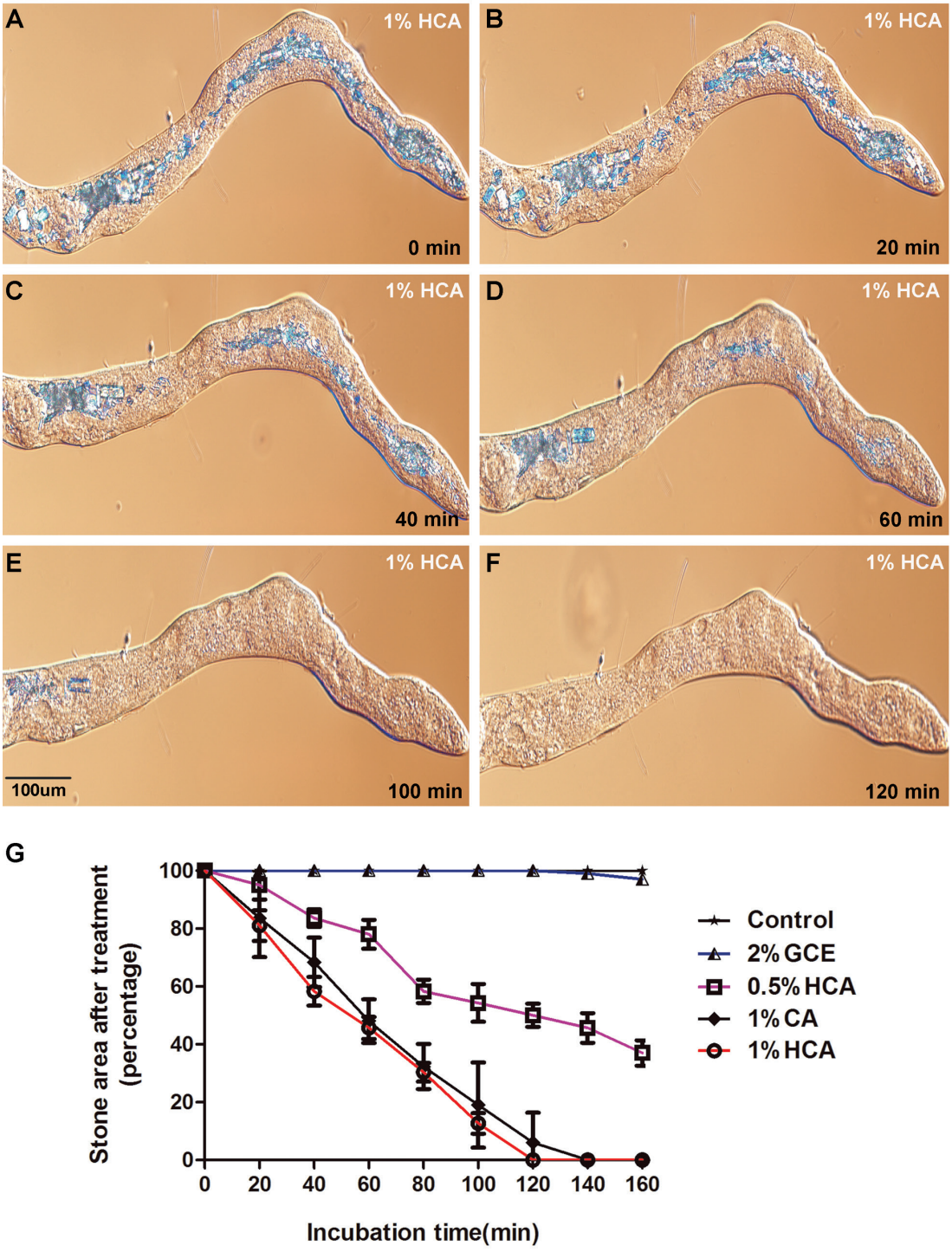

Figure 7. Hydroxycitrate directly dissolves calcium oxalate crystals in *Drosophila* renal tubules *ex vivo*. Images of renal calcium oxalate crystal dissolution in intact Malpighian tubules treated with HCA solutions *ex vivo* at different time points (**A-F**). Wild type flies were reared in fly food containing 0.3% NaOx for one week. Intact Malpighian tubules were dissected from wild type flies reared in fly food containing 0.3% NaOx and treated with HCA, CA or GCE solutions, and calcium oxalate crystal dissolution was monitored using live-imaging. Images were taken at 0 min (**A**), 20 min (**B**), 40 min (**C**), 60 min (**D**), 100 min (**E**), 120 min (**F**). The total area of calcium oxalate crystal was measured using cellSens software. Renal calcium oxalate crystal dissolution rate was calculated by dividing the remaining calcium oxalate crystal area by the total crystal area before treatment. The results are expressed as mean ± SD (n=3). **G,** HCA and CA efficiently dissolved calcium oxalate crystals within 120 minutes. Similar to control group (H_2O), GCE had no effect on dissolving calcium oxalate crystals. Same scale bars were used in **A-F** (100 μm).

in rat urolithiasis model. Our preliminary result showed that *Garcinia cambogia* extract can also efficiently dissolve calcium oxalate stones *in vivo*[25]. *Garcinia cambogia* extract, as a natural fruit product, has been used for weight loss for over 40 years. We really hope that our study could provide a solid foundation for clinical trials using *Garcinia cambogia* extract as a new medication for urolithiasis.

Conclusions

In summary, we demonstrate that clinical-grade *Garcinia cambogia* extract efficiently removes calcium oxalate stones in *Drosophila* Malpighian tubule *in vivo* and hydroxycitric acid directly dissolve calcium oxalate crystals in *Drosophila* renal tubules *ex vivo*. These data also suggests that clinical-grade *Garcinia cambogia* extract could be used to treat patients with genetic and non-genetic nephrolithiasis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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