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Quercetin inhibits calcium oxalate crystallization and growth but promotes crystal aggregation and invasion

Sakdithep Chaiyarit, Somsakul Phuangkham, Visith Thongboonkerd *

Medical Proteomics Unit, Research Department, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

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ABSTRACT

Recent evidence has shown an association between kidney stone pathogenesis and oxidative stress. Many anti-oxidants have been studied with an aim for stone prevention. Quercetin, a natural flavonol, is one among those eminent anti-oxidants with satisfactory anti-inflammatory property to cope with renal tissue injury in kidney stone disease. Nevertheless, its direct effect (if any) on calcium oxalate (CaOx) crystals and the stone formation mechanism had not been previously explored. This study has addressed the ability of quercetin at various concentrations (2.5, 5, 10, 20, 40, 80 and 160 μM) to directly modulate CaOx crystallization, growth, aggregation, adhesion on kidney cells, and invasion through the matrix. The data have shown that quercetin significantly inhibits CaOx crystallization and crystal growth but promotes crystal aggregation in concentration-dependent manner. However, quercetin at all these concentrations do not affect CaOx adhesion on kidney cells. For the invasion, quercetin at all concentrations constantly promotes CaOx invasion through the matrix without concentration-dependent pattern. These discoveries have demonstrated for the first time that quercetin has direct but dual modulatory effects on CaOx crystals. While quercetin inhibits CaOx crystallization and growth, on the other hand, it promotes CaOx crystal aggregation and invasion through the matrix. These data highlight the role for quercetin in direct modulation of the CaOx crystals that may intervene the stone pathogenesis.

1. Introduction

Calcium oxalate (CaOx) is the most prominent key element for kidney stone initiation and development (Aizezi et al., 2022). Formation of the kidney stone involves crystallization of CaOx neocrystals followed by the increase in size of crystalline mass by enlargement of individual crystals and/or their self-aggregation (Alexander et al., 2022). The crystals can adhere on kidney cells, leading to crystal retention that favors the stone development (Khan et al., 2016; Thongboonkerd, 2019). The crystals can also induce inflammation and renal tissue injury that, in turn, enhances crystal adhesion and retention on the kidney cells (Capolongo et al., 2023; Chaiyarit and Thongboonkerd, 2020; Khan et al., 2016; Thongboonkerd, 2019). Therefore, prevention of renal tubular cell injury together with reduction of crystallization, crystal enlargement, aggregation and adhesion may be able to prevent the stone formation.

The correlation between oxidative stress and kidney stone disease has been documented in both *in vitro* (Chaiyarit and Thongboonkerd, 2021; Gu et al., 2022; Peerapen et al., 2018) and *in vivo* (Gu et al., 2022; Kumar et al., 2023; Liu et al., 2023) evidence. Several previous investigations have illustrated that CaOx crystals can trigger oxidative stress (Vinaiphat et al., 2017; Xun et al., 2022), mitochondrial dysfunction (Chaiyarit and Thongboonkerd, 2012, 2020), and paracellular and tight junction defects (Hadpech et al., 2022; Peerapen and Thongboonkerd, 2011, 2013, 2021). Vice versa, increasing number of references have demonstrated that oxidative stress can enhance the intrarenal accumulation of CaOx crystals inside the tubular lumen and/or interstitial area that is one of the critical steps for kidney stone development (Khan et al., 2021; Xun et al., 2022; Ye et al., 2021). Imbalance of reactive oxygen species (ROS) production and elimination results in inflammatory response, defective immunity and tissue injury. Renal tubulointerstitial injury can trigger fibrotic process that activates extracellular matrix (ECM) secretion and accumulation in the renal tissue (Peerapen and Thongboonkerd, 2020; Shin et al., 2022; Yoodee et al., 2021). These matters together with calcium phosphate accumulation then induce Randall's plaque formation and ultimately the stone development (Evan et al., 2018; Khan et al., 2021; O'Kell et al., 2019).

Numerous anti-oxidative compounds have been recently applied to

* Corresponding author. Head of Medical Proteomics Unit, Research Department, Siriraj Hospital, Mahidol University, 6th Floor - SiMR Building, 2 Wanglang Road, Bangkoknoi, Bangkok, 10700, Thailand.

E-mail addresses: thongboonkerd@dr.com, vthongbo@yahoo.com (V. Thongboonkerd).

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prevent the stone formation (Hong and Qin, 2023; Jian et al., 2021; Peerapen and Thongboonkerd, 2023). Quercetin is one of the most abundant polyphenolic flavonols generally found in vegetables and fruits (Salehi et al., 2020). It can be detected in multiple organs/tissues, e.g., liver, lung, kidney, small intestine, brain, heart and spleen (Batiha et al., 2020; Salehi et al., 2020). Quercetin is known as a strong anti-oxidant that has been introduced to manage oxidative stress in many diseases (Batiha et al., 2020; Salehi et al., 2020). In kidney stone disease, quercetin has been shown to reduce oxidative stress, tissue injury and intrarenal CaOx deposition in animal models (Guzel et al., 2021; Park et al., 2008; Zhu et al., 2014). Nevertheless, it was unclear that whether quercetin has direct effect (if any) on CaOx crystals and stone formation mechanism. This study thus aimed to address its ability to directly modulate CaOx crystallization, growth, aggregation, adhesion on kidney cells, and invasion through the ECM.

2. Materials and methods

2.1. CaOx crystallization assay

This assay was done using previously described protocol (Thongboonkerd et al., 2006, 2008). Briefly, 0.5 ml of 10 mM calcium chloride in crystallization buffer (10 mM Tris-HCl and 90 mM NaCl; pH 7.4) was placed into each well of 24-well plate (Corning Inc.; Corning, NY). Two μl of quercetin (Sigma-Aldrich; St. Louis, MO) solubilized in dimethyl sulfoxide (DMSO) (Sigma-Aldrich) at various stock concentrations was added (to make its final concentration at 2.5, 5, 10, 20, 40, 80 or 160 μM). The wells added with 2 μl DMSO served as the negative control, whereas those added with 2 μl crystallization buffer alone served as the blank control. Thereafter, 0.5 ml of 1 mM sodium oxalate in crystallization buffer was gently added into each well. The mixture was incubated at 25 °C for 1 h, and the crystalline products were imaged under Eclipse Ti–S inverted phase-contrast microscope (Nikon; Tokyo, Japan). Crystal size was measured from at least 100 crystals/sample in ≥10 random fields/sample by using NIS Elements D software version 4.11 (Nikon).

2.2. CaOx crystal growth assay

This assay was done using previously described protocol (Amimanan et al., 2017; Khamchun et al., 2019). Briefly, 0.5 ml of 10 mM calcium chloride in crystallization buffer was placed into each well of 24-well plate. Then, 0.5 ml of 1 mM sodium oxalate in crystallization buffer was gently added, and the mixture was incubated at 25 °C for 1 h. At this time-point (T_0), the crystalline products were imaged using the inverted phase-contrast microscope. Two μl of quercetin in DMSO at various stock concentrations was added (to make its final concentration at 2.5, 5, 10, 20, 40, 80 or 160 μM). The wells added with 2 μl DMSO served as the negative control, whereas those added with 2 μl crystallization buffer alone served as the blank control. The mixture was then further incubated for 60 min. At this latter time-point (T_{60}), the crystal images were taken again. Crystal sizes at both T_0 and T_{60} were measured by using NIS Elements D software version 4.11. Δ Crystal size (representing crystal growth) was determined from at least 100 crystals/sample in ≥10 random fields/sample using the following formula.

Formula 1:

$$\Delta \text{ Crystal size } (\mu m^2) = \text{Crystal size at } T_{60} \ (\mu m^2) - \text{Crystal size at } T_0 \ (\mu m^2)$$

2.3. CaOx crystal aggregation assay

This assay was done using previously described protocol (Chaiyarit and Thongboonkerd, 2017; Kanlaya et al., 2019). Briefly, CaOx crystals were prepared as described above (10 mM calcium chloride was mixed 1:1, v/v, with 1 mM sodium oxalate in crystallization buffer). After incubation at 25 °C overnight, the mixture was centrifuged at 2000×*g* for 5 min to harvest the crystals. They were washed with methanol and allowed to air-dry. The crystals (1000 μg) were resuspended in 1 ml crystallization buffer in each well of 6-well plate (Corning Inc.). Two μl of quercetin in DMSO at various stock concentrations was added (to make its final concentration at 2.5, 5, 10, 20, 40, 80 or 160 μM). The wells added with 2 μl DMSO served as the negative control, whereas those added with 2 μl crystallization buffer alone served as the blank control. After incubation with continuous shaking at 300 rpm and 25 °C for 1 h in ThermoMixer shaking incubator (Eppendorf; Hauppauge, NY), the crystal images were taken using the inverted phase-contrast microscope. Number of the crystal aggregates ("clusters of ≥3 individual crystals" (Chaiyarit and Thongboonkerd, 2017)) was counted from at least 15 random fields/sample.

2.4. CaOx crystal-cell adhesion assay

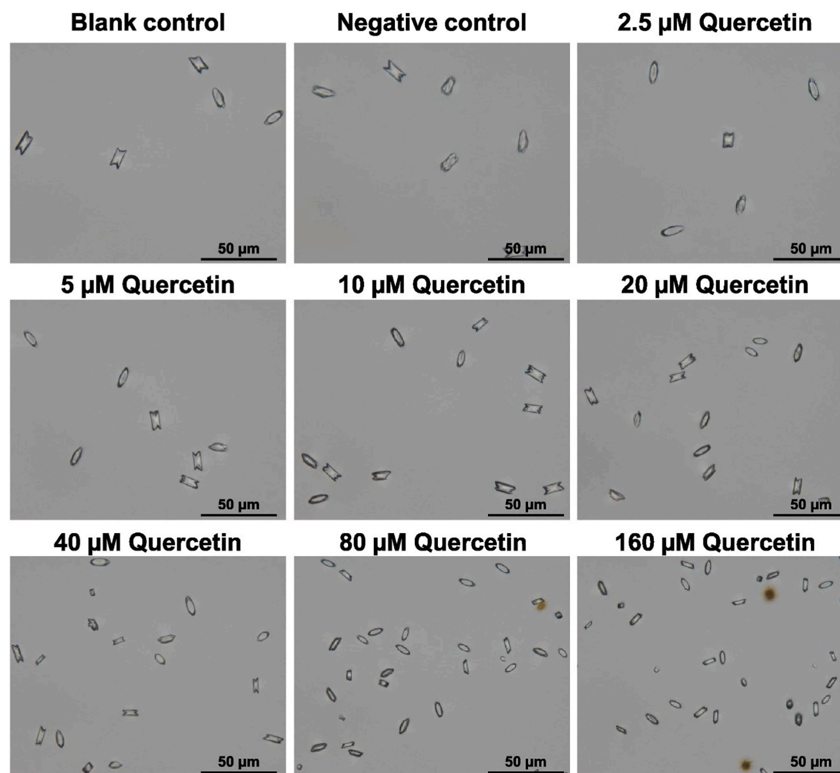
This assay was done using previously described protocol (Chiangjong and Thongboonkerd, 2012, 2016). Briefly, the CaOx crystals were prepared as described in the crystal aggregation assay and decontaminated by UV radiation for 30 min before use. MDCK kidney cells, initially derived from distal renal tubules of a dog (ATCC; Manassas, VA), were cultured in each well of 6-well plate (250,000 cells/well) and incubated at 37 °C with 5% CO_2 for 24 h. The culture medium was then replaced with the fresh one containing 100 μg/ml COM crystals. Two μl of quercetin in DMSO at various stock concentrations was added (to make its final concentration at 2.5, 5, 10, 20, 40, 80 or 160 μM). The wells added with 2 μl DMSO served as the negative control, whereas those added with 2 μl crystallization buffer alone served as the blank control. After 1-h incubation and five washes with PBS, the crystals adhered on the cells were then imaged using the inverted phase-contrast microscope and counted from at least 15 random fields/sample.

2.5. CaOx crystal invasion assay

This assay was done using previously described protocol (Chiangjong and Thongboonkerd, 2012, 2016). Briefly, 20 μg CaOx crystals (prepared as described above) were resuspended in 200 μl Eagle's minimum essential medium (Gibco; Grand Island, NY) and incubated overnight at 4 °C with 2 μl of quercetin in DMSO at various stock concentrations (to make its final concentration at 2.5, 5, 10, 20, 40, 80 or 160 μM), DMSO (negative control), bovine serum albumin (Sigma-Aldrich) (positive control), or crystallization buffer alone (blank control). Thereafter, the mixture was centrifuged at 2000×*g* and 4 °C for 5 min, and the crystal complexes were washed with PBS and further incubated with 200 μl of 0.3 pM Lys-plasminogen (Fitzgerald Industries international; Acton, MA) in PBS at 37 °C for 60 min. The excessive (unbound) plasminogen was removed by centrifugation at 2000×*g* and 4 °C for 5 min, and the crystal complexes were washed with PBS. Thereafter, 100 μl of 0.15 pM urokinase plasminogen activator (Fitzgerald Industries International) in PBS was added, and the crystal complexes were placed on-top of the matrix gel inside the ECM migration chamber. After 24-h incubation at 37 °C, the remaining crystal complexes were discarded. The crystals invaded inside the ECM chamber were imaged by using a microscope with differential interference contrast (DIC) mode (Eclipse 80i) (Nikon). Crystal invasion distance (*d*) was then measured from the top of the migration chamber to the front of the migrated CaOx crystals from at least 15 random fields/sample using NIS Elements D software version 4.11 (Nikon).

2.6. Statistical analysis

All measurable data were gained from three independent experiments using independent biological samples and are reported as mean ± SD. Differences among groups were statistically determined by one-



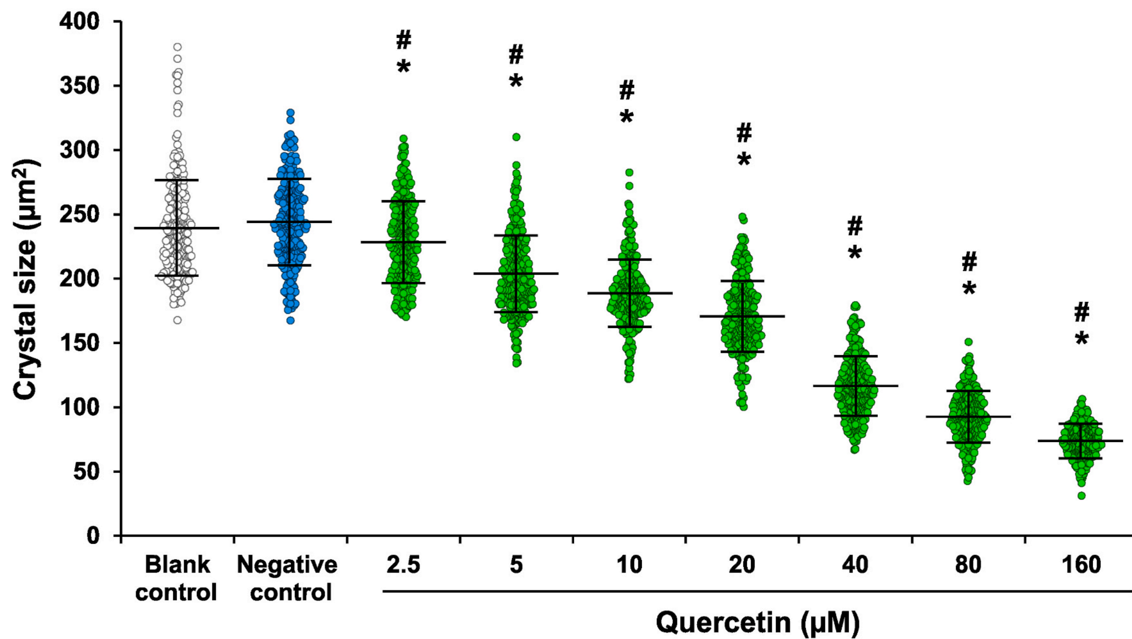


Fig. 1. Effect of quercetin on CaOx crystallization. Crystallization assay was performed with various final concentrations of quercetin, whereas DMSO served as the negative control and crystallization buffer alone served as the blank control. **(A):** Microscopic images of CaOx crystals after crystallization for 1 h. **(B):** Crystal size (mean ± SD) was measured from at least 100 crystals/sample in ≥10 random fields/sample. The data were derived from three independent experiments using independent biological samples. * $P < 0.05$ vs. the blank control; # $P < 0.05$ vs. the negative control.

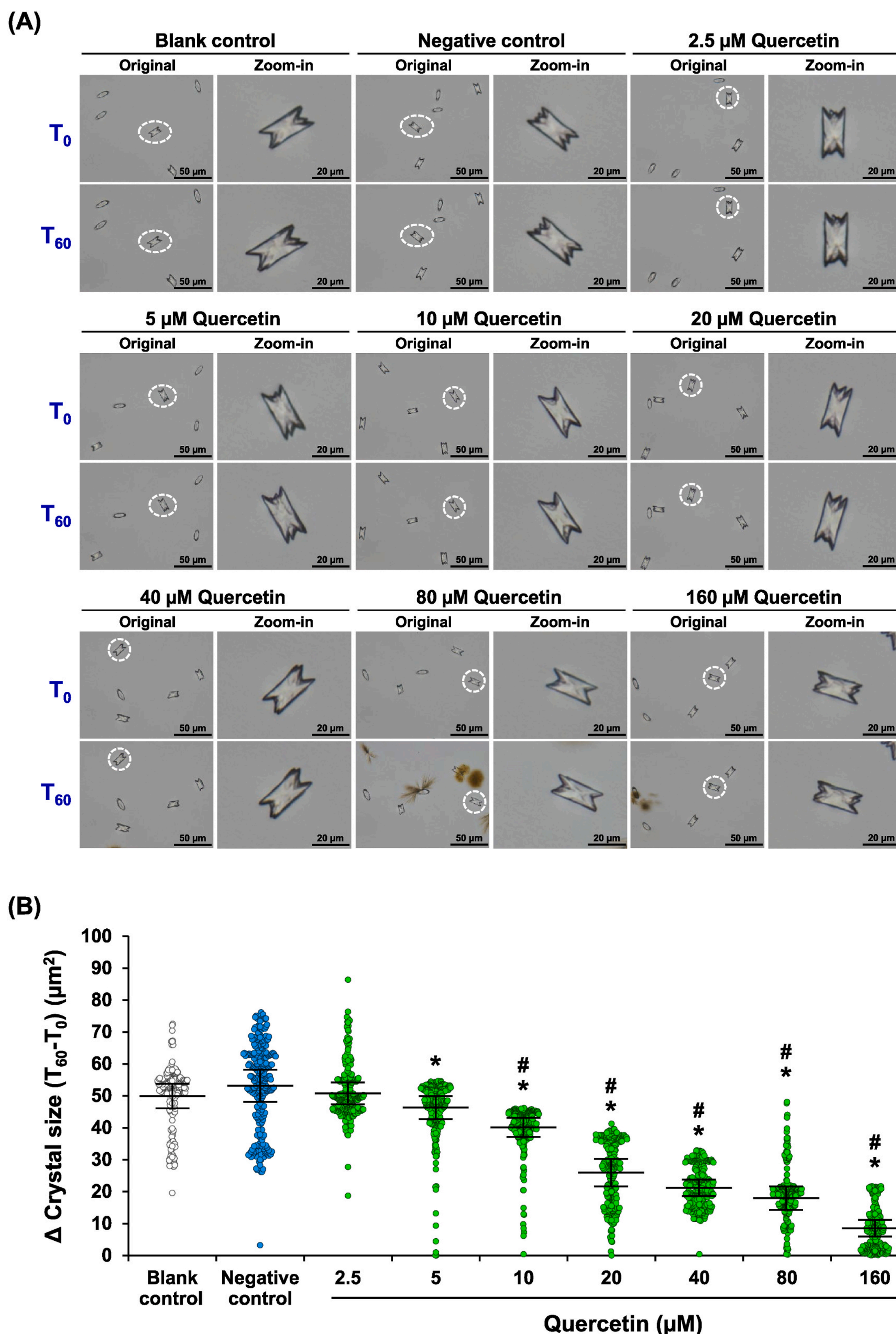
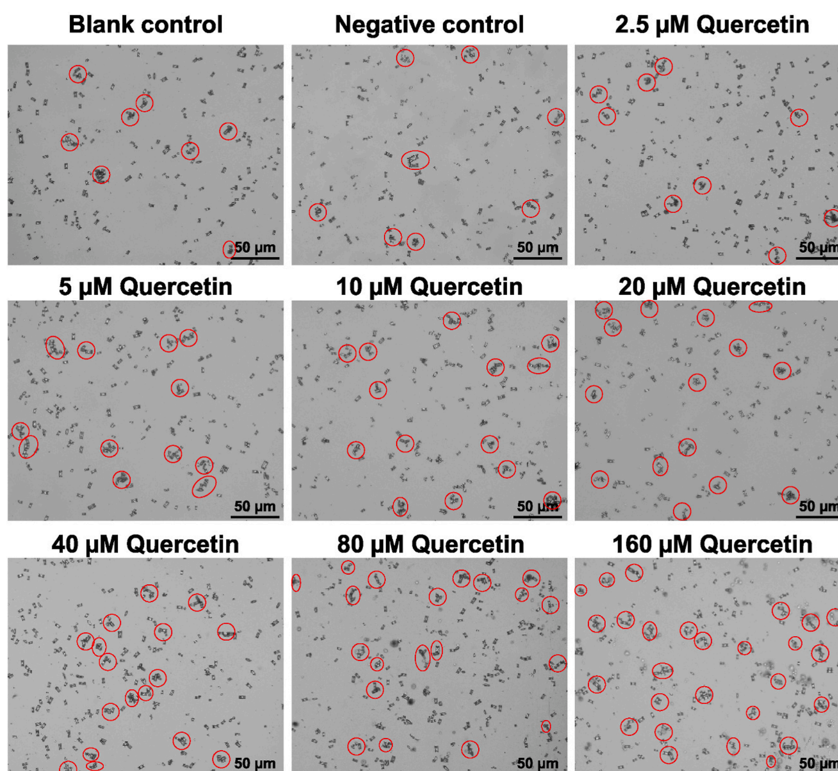


Fig. 2. Effect of quercetin on CaOx crystal growth. Crystal growth assay was performed with various concentrations of quercetin, whereas DMSO served as the negative control and crystallization buffer alone served as the blank control. **(A):** Microscopic images of CaOx crystals at T_0 (after initial crystallization) and T_{60} (after allowing the crystals to further enlarge for 60 min). Zoom-in image was taken from the area labeled with white dashed circle in the original image. **(B):** Crystal sizes (mean ± SD) at both T_0 and T_{60} were measured, and Δ crystal size (representing crystal growth) was determined from at least 100 crystals/sample in ≥10 random fields/sample using *Formula 1* (see Materials and methods). The data were derived from three independent experiments using independent biological samples. * $P < 0.05$ vs. the blank control; # $P < 0.05$ vs. the negative control.



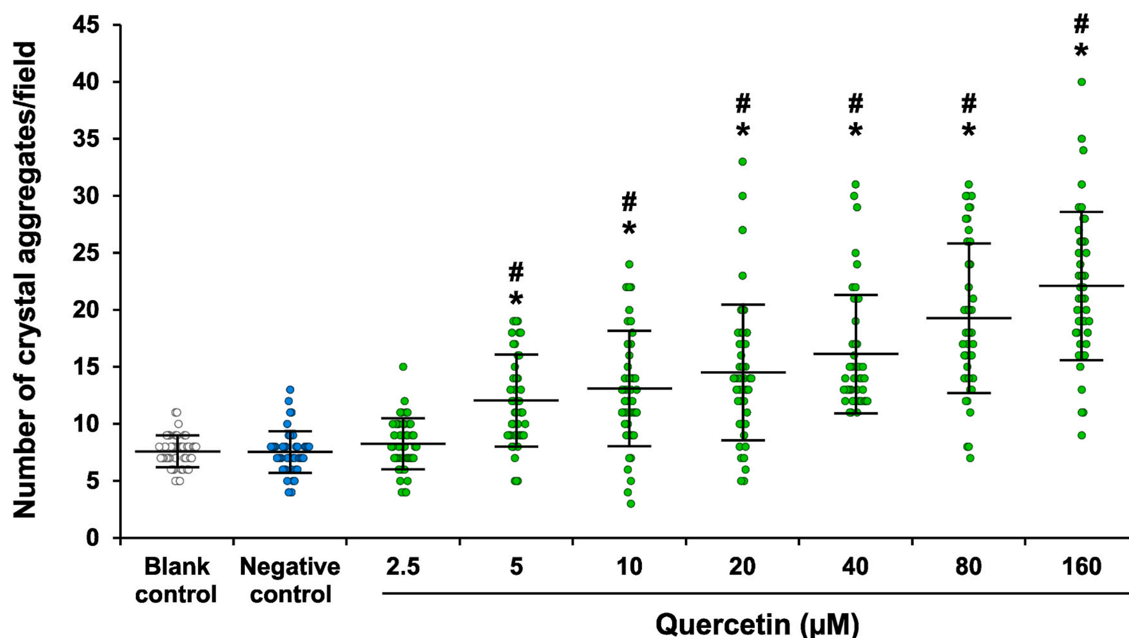
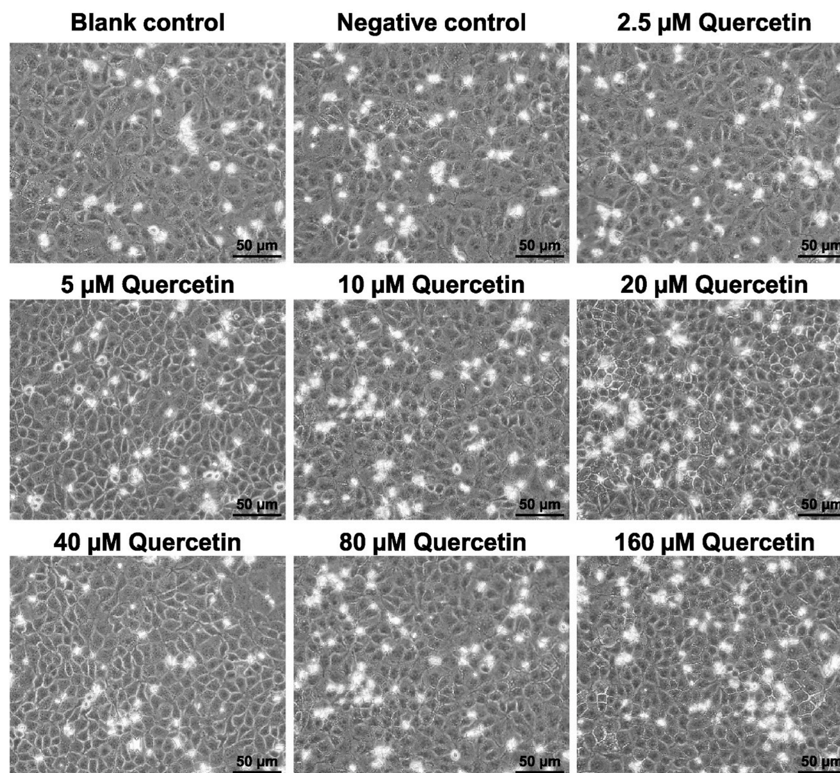


Fig. 3. Effect of quercetin on CaOx crystal aggregation. Crystal aggregation assay was performed with various concentrations of quercetin, whereas DMSO served as the negative control and crystallization buffer alone served as the blank control. **(A):** Microscopic images of CaOx crystal aggregates ("clusters of ≥3 individual crystals" (Chaiyarit and Thongboonkerd, 2017)) (labeled with the red dashed circles) after incubation for 1 h. **(B):** Number of crystal aggregates (mean ± SD) was counted from at least 15 random fields/sample. The data were derived from three independent experiments using independent biological samples. * $P < 0.05$ vs. the blank control; # $P < 0.05$ vs. the negative control. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



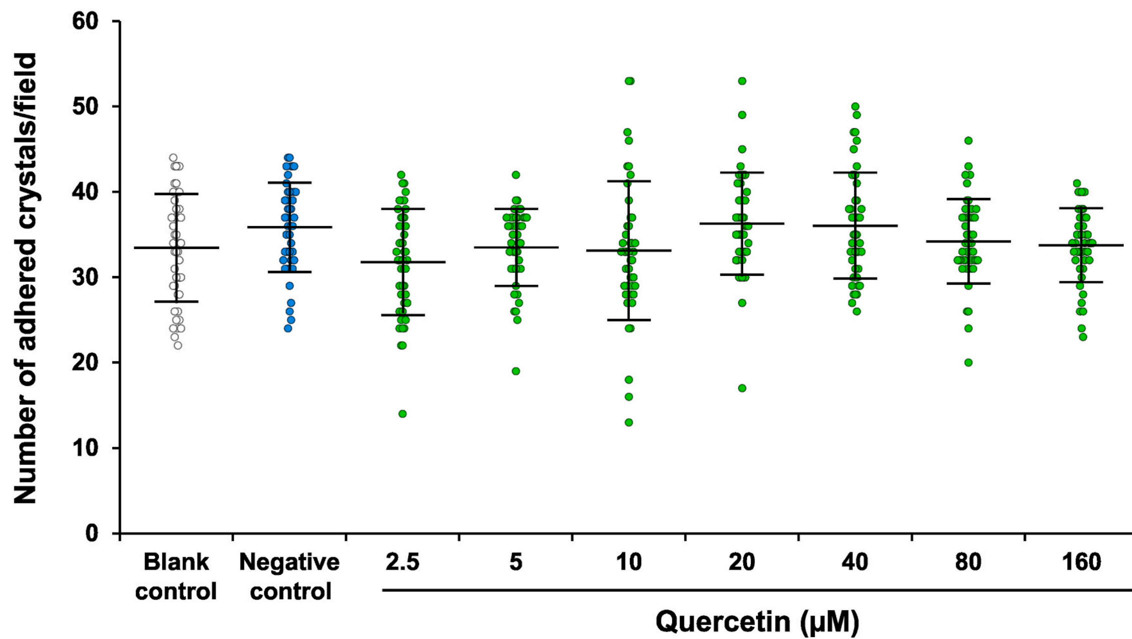
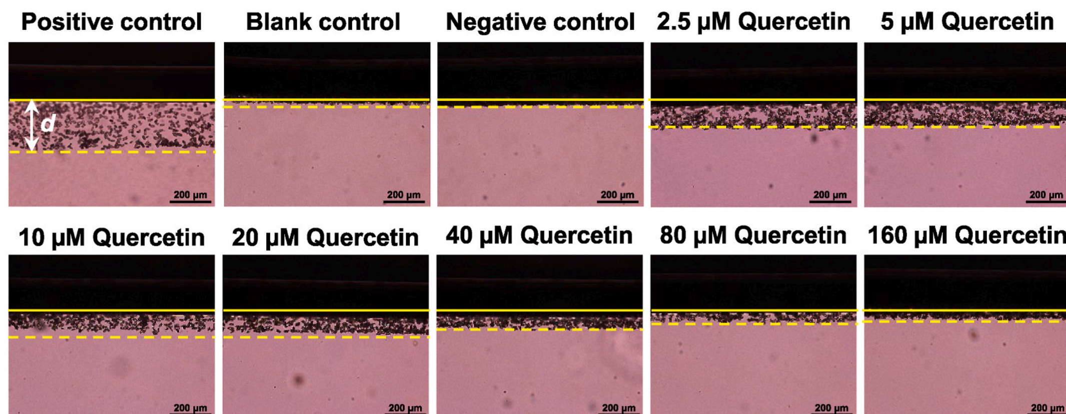


Fig. 4. Effect of quercetin on CaOx crystal adhesion on kidney cells. Crystal adhesion assay was performed on MDCK cells with various concentrations of quercetin, whereas DMSO served as the negative control and crystallization buffer alone served as the blank control. **(A):** Microscopic images of CaOx crystals remained on the cell monolayer after incubation with the crystals for 1 h followed by rigorous washes. **(B):** Number of the adhered crystals (mean ± SD) was counted from at least 15 random fields/sample. The data were derived from three independent experiments using independent biological samples. No significant difference among groups was observed.



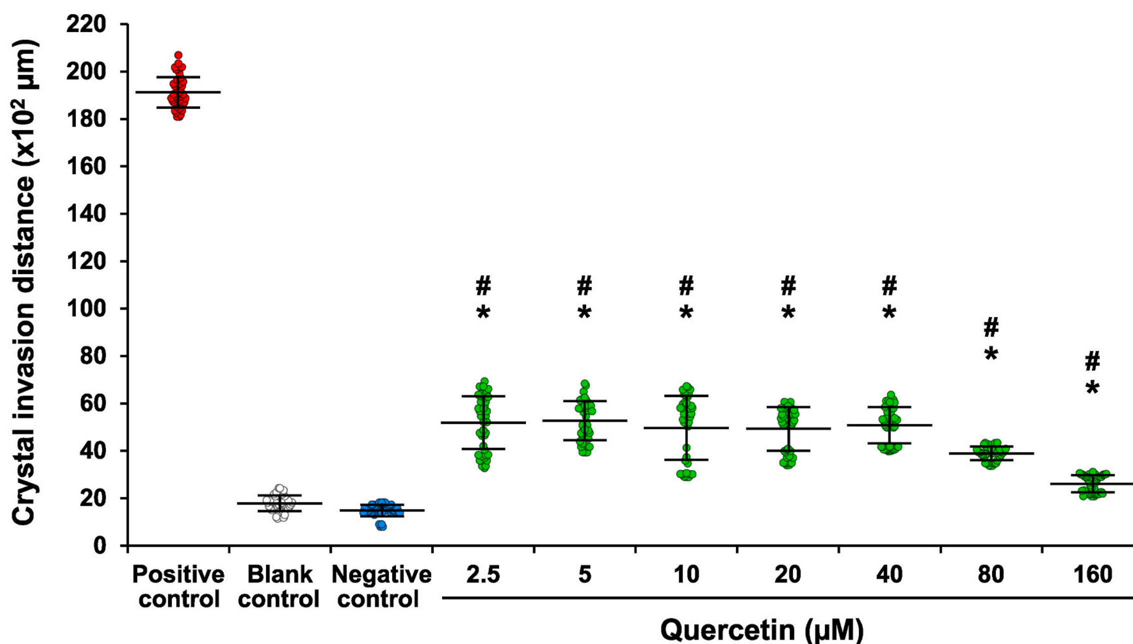


Fig. 5. Effect of quercetin on CaOx crystal invasion. Crystal invasion assay was performed in the ECM migration chamber with various concentrations of quercetin, whereas BSA served as the positive control, DMSO served as the negative control, and crystallization buffer alone served as the blank control. **(A):** Microscopic images of CaOx crystals migrated in the ECM chamber after 24-h incubation. The front of the migrated CaOx crystals is indicated as the horizontal dashed line in yellow, whereas the top of the migration chamber is indicated by the horizontal solid line in yellow. **(B):** Crystal invasion distance (labeled on the positive control, as an example, in panel **(A)** by the vertical two headed arrow in white with letter "*d*") (also applied to other conditions) was measured from at least 15 random fields/sample using NIS Elements D software version 4.11 (Nikon) and is reported as mean ± SD. The data were derived from three independent experiments using independent biological samples. * $P < 0.05$ vs. the blank control; # $P < 0.05$ vs. the negative control. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

way analysis of variance (ANOVA) with Tukey's post-hoc test for multiple comparisons using SPSS software (version 18.0) (IBM SPSS; Armonk, NY). $P < 0.05$ was set as the threshold for significant differences.

3. Results

To elucidate the direct effect of quercetin on CaOx crystals and to reflect its effect on kidney stone formation, quercetin was tested at various concentrations using a series of crystal assays. Additionally, crystal-cell interaction and invasion through the matrix were also examined. Range of its concentrations used in this study (2.5–160 μM)

was based on human blood and urine levels of quercetin reported in recent studies (Boonyong et al., 2023; Salehi et al., 2020). In parallel, the samples added with DMSO and crystallization buffer alone served as the negative control and blank control, respectively. CaOx crystallization assay, which can reflect nucleation of the initial stone development, was performed, and crystal size was measured from at least 100 crystals/sample in ≥10 random fields/sample. The findings showed that quercetin started to significantly reduce CaOx crystal size at the lowest concentration (2.5 μM) compared with the blank and negative controls (Fig. 1). Its higher concentrations exhibited greater inhibitory activity against the crystallization in a concentration-dependent manner (Fig. 1).

To evaluate the effect of quercetin on CaOx crystal enlargement, the crystal growth assay was then performed. CaOx crystal images at T_0 and T_{60} were taken and their differential size (Δ crystal size, representing growth of the crystals) was analyzed from at least 100 crystals/sample in ≥10 random fields/sample using *Formula 1* (see **Materials and methods**). The results demonstrated that quercetin started to significantly reduce Δ crystal size at 5 μM compared with the blank and negative controls (Fig. 2). Its higher concentrations exhibited greater inhibitory activity against the crystal growth in a concentration-dependent manner. Note that the significant effect of quercetin was not recognized at the lowest concentration (2.5 μM) (Fig. 2).

The degree of CaOx crystal aggregation was determined by counting the number of the crystal aggregates, which have been defined as "clusters of ≥3 individual crystals" (Chaiyarit and Thongboonkerd, 2017). The analysis revealed that quercetin started to significantly increase number of the crystal aggregates at 5 μM compared with the blank and negative controls (Fig. 3). Its higher concentrations exhibited greater promoting activity against the crystal aggregation in a concentration-dependent manner. Similar to the crystal growth assay, there was no significant effect of quercetin found at the lowest concentration (2.5 μM) (Fig. 3).

In addition, we investigated the effect of quercetin on CaOx crystal adhesion on the kidney cells. After incubation of MDCK cells with CaOx crystals, the unadhered crystals were removed, whereas the adhered crystals were counted for comparative analysis. The data demonstrated that quercetin at all concentrations did not alter the number of the adhered crystals as compared with the negative and blank controls (Fig. 4).

Moreover, CaOx crystal migration through the renal interstitium rich with the ECM (a mechanism of which may link between intratubular and interstitial stone formation mechanisms) was then evaluated. After laying the crystal complexes on top of the ECM migration chamber, the excess crystal complexes were removed, whereas the crystal invasion distance (*d*) was measured from at least 15 fields/sample. Fig. 5A showed the migrated CaOx crystals inside the ECM migration chamber. Measurements revealed that quercetin at all concentrations significantly increased the crystal migration distance compared with the blank and negative controls (Fig. 5B). However, such increase was not concentration-dependent (Fig. 5B).

4. Discussion

Natural food compounds and related products have been continuously investigated for their potential to be used as the novel therapeutic strategies in several diseases, especially those induced by oxidative stress and inflammation (Luo et al., 2023; Mirmohammadali and Rosenkranz, 2023; Nisar et al., 2023). Plant-based anti-oxidants, such as vitamin E (Nisar et al., 2023), catechin (Baranwal et al., 2022; Talebi et al., 2021), caffeic acid (Ganguly et al., 2023; Pandey et al., 2023), gallic acid (Bhuia et al., 2023; Keyvani-Ghamsari et al., 2023), resveratrol (Bi et al., 2023; Ramli et al., 2023), diosmin (Gerges et al., 2022; Mustafa et al., 2022) and quercetin (Shabir et al., 2022; Zhou et al., 2023), have been shown to rescue the oxidative stress conditions by scavenging ROS and their intermediates, reducing localized oxygen levels, chelating metal ion, and inhibiting lipid peroxidation. They have been applied for prevention of several diseases, e.g., neurodegenerative disorders, cardiovascular diseases, atherosclerosis, diabetes, and cancers (Baranwal et al., 2022; Bhuia et al., 2023; Bi et al., 2023; Ganguly et al., 2023; Gerges et al., 2022; Keyvani-Ghamsari et al., 2023; Luo et al., 2023; Mirmohammadali and Rosenkranz, 2023; Mustafa et al., 2022; Nisar et al., 2023; Pandey et al., 2023; Ramli et al., 2023; Shabir et al., 2022; Talebi et al., 2021; Zhou et al., 2023). In addition, the protective roles of many polyphenols have been investigated in kidney stone disease (Ahmed et al., 2018; Hong and Qin, 2023). However, the molecular mechanisms underlying their preventive effects against CaOx stone formation remains poorly understood.

The link between oxidative stress and kidney stone pathogenesis has been clearly demonstrated by many previous studies (Albert et al., 2020; Chaiyarit and Thongboonkerd, 2020; Khan et al., 2021). Several types of anti-oxidative compounds have been investigated in cell culture and animal studies (Hong and Qin, 2023; Jian et al., 2021; Peerapen and Thongboonkerd, 2023). Quercetin is one among those anti-oxidants that may have a potential in kidney stone prevention (Guzel et al., 2021; Park et al., 2008; Zhu et al., 2014). Thus, several studies have focused more attention on quercetin and its pharmacokinetics, toxicity and biological activities, especially for prevention of kidney stone disease (Guzel et al., 2021; Park et al., 2008; Zhu et al., 2014). Although quercetin has been well documented for preventing the stone initiation and development by reducing oxidative stress and kidney injury, its precise molecular mechanism and direct effects on CaOx crystals were previously ambiguous and needed further elucidations.

Herein, we address the direct modulatory effects of quercetin on CaOx crystals in the stone formation processes. Quercetin inhibited CaOx crystallization and growth in a concentration-dependent manner. By its chelating property, quercetin might compete with oxalate to bind free calcium ions, leading to the reduction of core materials for CaOx crystal formation and enlargement. Degrees of the declines of crystallization and crystal growth were greater when the concentrations of quercetin increased. These concentration-dependent inhibitory effects might be the direct mechanism to hamper development and slow progression of kidney stone. However, the inhibitory effects of quercetin seems to be less potent than those of the well-known strong calcium-chelating agents, such as ethylenediaminetetraacetic acid (EDTA), sodium citrate and potassium citrate (Carvalho et al., 2017; Choi and Zhong, 2020; Chutipongtanate et al., 2012; Doizi et al., 2018). Nevertheless, using the natural compound should be safer as it may introduce fewer toxic effects as compared with the more potent calcium-chelating chemicals, particularly EDTA. Comparing effects of these chemicals deserves further investigations in the *in vivo* setting.

On the other hand, we demonstrated that quercetin promoted CaOx crystal aggregation in a concentration-dependent manner. Based on its four calcium-binding sites, like in other flavonoids (Yi et al., 2021), quercetin might bind to the calcium molecules on the CaOx crystal surface and become the bridge to attract individual CaOx crystals to join together. Formation of the crystal aggregates is harmful to the kidney as they are more difficult to be eliminated via urination. Moreover, the aggregates can introduce greater degree of toxicity and renal tissue injury as compared with the isolated crystals, thereby promoting the stone development and progression (Sun et al., 2017).

Likewise, quercetin promoted crystal invasion through the matrix. However, this effect of quercetin was not concentration-dependent. It was possible that much lower concentration of quercetin (much lower than 2.5 μM) was sufficient to promote the crystal invasion. This hypothesis is consistent with the previously reported data indicating that quercetin has a high affinity to bind the urokinase plasminogen activator (Pavlovic et al., 2022; Xue et al., 2017), which is required for crystal invasion through the matrix (Chiangjong and Thongboonkerd, 2012, 2016). Therefore, there was no concentration-dependent effect observed at the greater concentrations.

Generally, a modulator can affect (either inhibit or promote) crystal adhesion on the kidney cells by competitive binding to the crystal

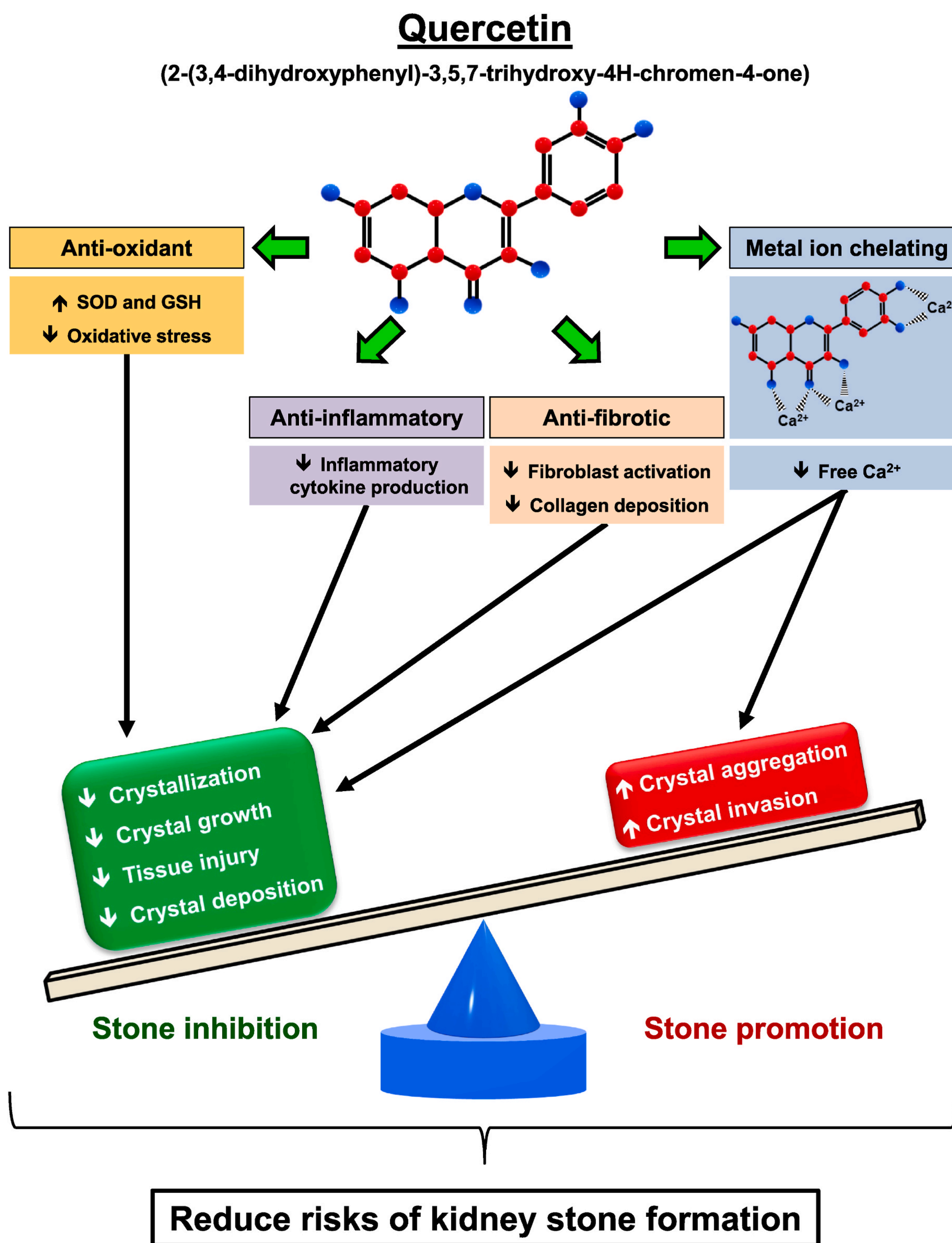


Fig. 6. Schematic summary of all the effects of quercetin on kidney stone formation. While quercetin inhibits CaOx crystallization and crystal growth in a concentration-dependent manner, quercetin concentration-dependently promotes CaOx crystal aggregation. Quercetin also promotes CaOx crystal invasion through the matrix, but without the concentration-dependent pattern. Although quercetin exerts the dual modulatory effects on CaOx crystals, its strong anti-oxidative activities, anti-inflammatory responses, and anti-fibrotic properties have been well-documented in several recent studies (Guzel et al., 2021; Liu et al., 2019; Salehi et al., 2020; Xiao et al., 2020). Moreover, the calcium-binding and chelating properties of quercetin have been previously reported (Ferlemi et al., 2016; Manman et al., 2019). Consequently, quercetin likely serves as an inhibitor, rather than a promoter for CaOx stone formation, considering these various different angles. SOD, superoxide dismutase; GSH, glutathione.

surface (Hadpech et al., 2023; Khamchun et al., 2019; Noonin et al., 2022) or alterations in the surface molecules on the kidney cells that serve as the crystal receptors (Peerapen et al., 2022; Peerapen and Thongboonkerd, 2016; Sueksakit and Thongboonkerd, 2019). It was surprising that while quercetin affected CaOx crystallization, growth, aggregation and invasion, it had no significant effect on crystal adhesion on kidney cells as compared with the blank and negative controls.

Our results showed that quercetin had direct but dual modulatory effects on CaOx crystals. While it inhibited CaOx crystallization and growth, quercetin promoted CaOx aggregation and invasion. The dual modulatory effects on CaOx have been shown in not only quercetin but also other phytochemicals and proteins. For example, diosmin reduces CaOx crystal size, enlargement, adhesion on kidney cells, and engulfment into the kidney cells, but induces crystal invasion and aggregation (Khamchun et al., 2021). An ECM protein, namely fibronectin, also exerts the dual modulatory activities on the CaOx crystals. While it reduces CaOx crystal mass, enlargement, and adhesion on the kidney cells, fibronectin enhances CaOx self-aggregation and invasion (Khamchun et al., 2019). In addition to phytochemicals and modulatory proteins, kidney cell membranes and their fragments also show such dual modulatory activities on the CaOx crystals. The cell membranes and their fragments reduce CaOx crystal size, but increase the crystal self-aggregation (Chutipongtanate and Thongboonkerd, 2010). Indeed, the overall modulatory effect of all these dual modulators of CaOx kidney stone relies heavily on their net outcome (after balancing such dual modulatory activities). Also, concentration/dosage, duration of administration and other several factors must be taken into account for considering the overall outcome of all the dual modulators. Although we have shown herein that quercetin exerts the dual modulatory effects on CaOx crystals, its strong anti-oxidative activities, anti-inflammatory responses, and anti-fibrotic properties have been well-documented in several previous studies (Guzel et al., 2021; Liu et al., 2019; Salehi et al., 2020; Xiao et al., 2020). Moreover, the calcium-binding and chelating properties of quercetin have been previously reported (Ferlemi et al., 2016; Manman et al., 2019). As the net outcome, quercetin likely serves as an inhibitor, rather than a promoter, for CaOx stone formation considering these various different angles (Fig. 6).

In summary, this study has demonstrated the direct modulatory effects of quercetin on CaOx crystals in the stone formation processes. The results have demonstrated that quercetin has the dual modulatory effects on CaOx crystals. While it inhibits CaOx crystallization and crystal growth in a concentration-dependent manner, quercetin concentration-dependently promotes CaOx crystal aggregation. Quercetin also promotes CaOx crystal invasion through the matrix, but without the concentration-dependent pattern. These data highlight the role for quercetin in direct modulation of the CaOx crystals that may intervene the stone pathogenesis.

CRediT authorship contribution statement

Sakdithep Chaiyarit: Designed research, Performed experiments, Formal analysis, Analyzed data, Wrote the manuscript; All authors reviewed and approved the manuscript. **Somsakul Phuangkham:** Designed research, Performed experiments, Formal analysis, Analyzed data. **Visith Thongboonkerd:** Designed research, Formal analysis, Analyzed data, Wrote the manuscript; All authors reviewed and approved the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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