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Review article

The fruit fly kidney stone models and their application in drug development

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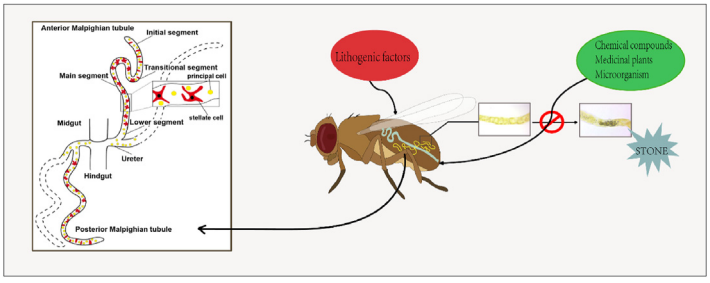
HIGHLIGHTS

- Kidney stone disease is a global problem affecting about 12% of the world population.
- The fruit fly kidney stone models were established via dietary or genetic methods.
- New antilithogenic leads can be identified using fruit fly models.

GRAPHICAL ABSTRACT



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ABSTRACT

Kidney stone disease is a global problem affecting about 12% of the world population. Novel treatments to control this disease have a huge demand. Here we argue that the fruit fly, as an emerging kidney stone model, can provide a platform for the discovery of new drugs. The renal system of fruit fly (Malpighian tubules) is similar to the mammalian renal tubules in both function and structure. Different fruit fly models for different types of kidney stones including calcium oxalate (CaOx) stones, xanthine stones, uric acid stone, and calcium phosphate (CaP) stones have been successfully established through dietary or genetic approaches in the last ten years, notably improved our understanding of the formation mechanisms of kidney stone diseases. The fruit fly CaOx stones model, which is mediated by treatment with dietary lithogenic agents, is also one of the most potential models for drug development. Various potential antilithogenic agents have been identified using this model, including new chemical compounds and medicinal plants. The fruit fly kidney stone models also afford opportunities to study the therapeutic mechanism of these drugs in deeper.

1. Introduction

Kidney stone disease (Nephrolithiasis) refers to solid objects formation within the renal pelvis and tubules by supersaturation of solutes in the urine [1]. The incidence of kidney stones disease is rising globally, now affecting about 12% of the world population [2]. The male to female ratio of patients with kidney stones is approximately 3:1 [3]. According to the classification of stone components, kidney stone can be divided into different types, including calcium oxalate (CaOx) stones, calcium phosphate (CaP) stones, uric acid (UA) stones, cystine stones, struvite stones, etc [4]. Of these, CaOx stone is the most common type. A clinical survey of patients with kidney stones showed that 79% of patients had

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CaOx stones, 11% had UA stones and 10% had other stones [3]. A variety of factors are contributing to the onset of CaOx stone diseases, such as genetics, diet, medications, urine pH, hypercalcemia, and hyperoxaluria [4,5]. The major medical treatments for kidney stones are physical methods, such as laser, shock wave, and percutaneous, to break the stones [6]. However, these surgical methods may damage the surrounding organs, and the recurrence rate is higher than 50% [7]. Post-surgery medical treatment to reduce the recurrence possibility would be necessary. But in fact, adverse gastrointestinal reactions and poor patient compliance with existing drugs make long-term prevention of stones recurrence difficult [8]. The development of new drugs with higher effectiveness and safety for kidney stone control is in huge demand.

The Rodents like rats and mice are frequently used as model animals to study kidney stones disease. CaOx kidney stones can be induced in rats by intraperitoneal injection of sodium oxalate (NaOx) or addition of other lithogenic agents including NaOx, glycolic acid, ethylene glycol (EG), and hydroxy-L-proline (HLP) to drinking water. Genetically modified mice are popularly used to study the genetic mechanism of stone formation. For example, Tamm-Horsfall protein knockout mice have crystals in medullar collecting ducts [9]. A nonnegligible shortcoming is the high costs and technical difficulties of making transgenic mice. Other issues like high breeding costs, long experimental periods, and ethical issues also limit the development and application of rodent kidney stone models [10]. Therefore, quicker, cost-limited, high--throughput screening platforms are needed to assist the search for novel drugs to treat kidney stones. Mounting evidence highlighted the emerging ability of small animals such as fruit flies and zebrafish for this requirement. The zebrafish is a popular model animal for toxicity studies [11], the application of zebrafish in kidney disease research was already summarized in the review written by Poureetezadi and Wingert in 2016 [12]. Here we review several informative fruit fly models of kidney stone disease developed over the past decade and their applications in drug development.

Fruit fly (*Drosophila melanogaster*) has received widespread attention as a model animal [13]. There is a high degree of gene conservation between the fruit fly and humans, 75% of human disease-related genes have corresponding genes in the fruit fly [14]. Compare to the rodent models, low breeding costs, short experimental period, well-understood genome, and abundant genetic modification techniques make the fruit fly a promising animal model for both genetic fundamental studies and large scale *in vivo* drug screening [14].

The renal system of the fruit fly is composed of the nephrocytes and the Malpighian tubules (MTs) [15]. Nephrocytes are present around the heart and the esophagus to remove the waste from the hemolymph like the human glomerulus. The MTs are functionally similar to the tubular parts of the nephron, producing urine and keeping fluid homeostasis via active transport of water, ions, and organic solutes between the hemolymph and the lumen of MTs [13,16]. The structure of fruit fly MTs is simple and the tubules can be dissected easily and completely. The MTs are transparent and stone formation can be observed under a light microscope without any subsequent treatment [17]. The fruit fly kidney stone models have been developed by dietary supplementation and genetic manipulation separately. Adult fruit flies supplemented with NaOx, EG, HLP for two weeks resulted in CaOx crystals formation in the MTs of fruit fly separately [18]. In another study, knocking down *dAGXT* contributed to the CaOx stone formation [19]. The UA stone model was established by down-regulating the function of *Uro* [20]. The CaP stone model in fruit fly relies on exogenous administration of sodium phosphate [21]. The fruit fly serves as a perfect platform for studying the pathogenesis of kidney stones and the high-throughput primary screening of novel drugs candidates. In this review, we first compared the fruit fly renal system with the human kidney from morphology and molecular working models. We next introduced the newly developed fly kidney stone models over the past decade including the establishment methods, important results of genetic studies as well as the reported drug development research using these models. As a conclusion, we point out that the fruit fly kidney stone models can supply quicker, cost-limited, high-throughput screening platforms for the development of novel drugs to treat kidney stones.

2. The fruit fly renal system

2.1. General morphology

Fruit fly MTs are similar to the mammalian renal tubules in both function and structure. They filter hemolymph and remove metabolic waste to adjust the balance of water, osmotic pressure, electrolyte, and organic solute in the fruit fly's body [16]. There are four MTs in the fruit fly, formed separately the anterior and posterior pairs. Both tubules pairs join to form two ureters, which connect with the anterior end of the hindgut, which directs joints to the posterior end of the midgut [22]. The MTs generate urine through the active transport of ions, water, and organic solutes from the hemolymph to the lumen of the MTs [23]. The MTs can be divided into five different regions including initial, transitional, main, lower segments, and the ureter. The MTs are composed of two types of cells, principal cells and stellate cells (Figure 1A) [13,22]. They correspond to the principal and intercalated cells of mammalian renal tubules [22]. The principal cells (PCs) are big epithelial cells with regular form. They transport cations and organic solutes [6]. The stellate cells (SCs) have a star-like morphology and are regularly embedded in the PC-formed tubules, which are responsible for the chloride ions and water transport [6]. These two cell types cooperate for urine production and the metabolic waste filtration functional analogy to the human glomerulus.

2.2. Water balancing and ion transport

The H^+ transporter vacuolar proton ATPase (V-ATPase) on the apical surface of PCs pumps H^+ from the cells to the lumen of MTs [24]. The H^+ in the lumen comes back via H^+/K^+-ATPase while the intracellular potassium ions are pumped to the lumen, thereby establishing an electrochemical gradient. This gradient drives the chloride ions from hemolymph into the lumen of MTs through the chloride conductance channel ClC-a in SCs [25]. The inwardly rectifying potassium channel Irk transports K^+ from the hemolymph to the cells [26]. The basolateral localized Na^+/K^+-ATPase exchanger and Na-dependent Cl^-/HCO_3^- exchanger, as well as the apical localized Na^+/H^+ exchanger Nha1 and Nha2, are also involved in the maintaining of Na^+, K^+ and Cl^- balance between the hemolymph, Malpighian cells and the lumen of tubules, thus regulate the water secretion and re-absorption during urine producing [27]. In SCs, the ClC-a maintains fluid balance by transporting Cl^- from hemolymph to lumen [28]. These ion transports actively make a stable concentration gradient of ions between the lumen and the hemolymph, thus creating an osmotic potential to promote the water fluid from hemolymph to lumen. The water comes into the lumen through the *Drosophila* aquaporin (Drip), which is localized on the cell membrane of stellate cells [25](Figure 1B).

In addition, fruit fly MTs regulate the calcium, magnesium, phosphorus, and oxalate levels in the body. These ions directly affect the stone formations. The Ca^{2+} transport mainly occurs in the initial/transition section of the MTs. The Mg^{2+} transport is implicated in fruit fly transient receptor potential cation channel, subfamily M (TRPM), expresses in the initial/transitional segment. Major facilitator superfamily 13 (MFS13) transport phosphate (Pi) [27]. The *prestin* encodes a protein homologous to the human Slc26a5 and Slc26a6 transporters, and mediates the exchange of Cl^- with a broad range of anion substrates, such as $oxalate^{2-}$, CO_3^{2-}, SO_4^{2-}, $S_2O_3^{2-}$ and $formate^-$ [29]. Knockdown of *prestin* in the MTs resulted in a significant reduction of CaOx stones formation [30]. In another study, it was found that SO_4^{2-} and $S_2O_3^{2-}$ competitively inhibited the transport of oxalate by dPrestin and could inhibit the CaOx stones formation [31]. Figure 1B showed most of the important transporters in

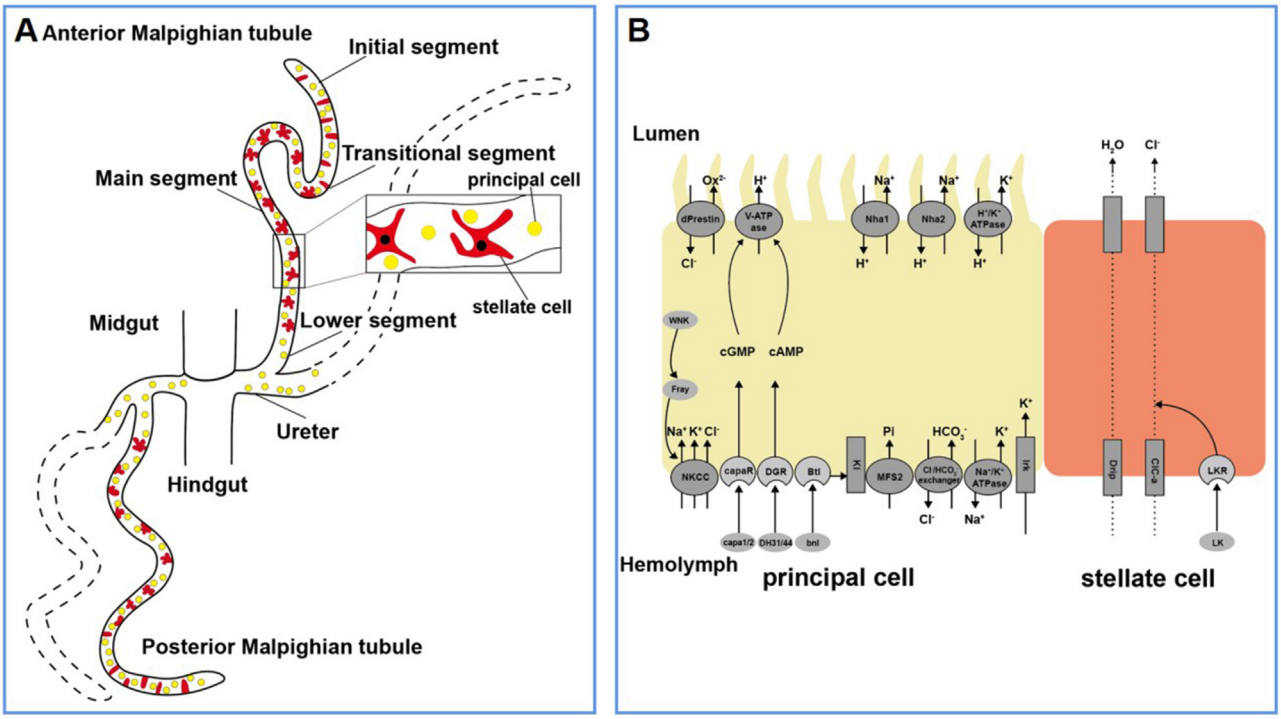

Figure 1. The structure and working principle of fruit fly Malpighian tubules. (A) Classical morphology of the adult fruit flies MTs. (B) Most of the important transporters in PCs and SCs and the working model for maintaining water and ions balancing in fruit fly MTs.

PCs and SCs and their working model for maintaining water and ions balancing in fruit fly MTs.

2.3. The regulation of ion and water transport

The ion and water transport in MTs is controlled extracellularly by hormones from the hemolymph, as well as intracellularly by activation of certain membrane transporters. Capability peptides 1/2 (capa1/2) and diuretic hormone 31/44 (DH31/44) stimulate their receptors located on the basal of PCs and then mobilize the secondary messenger cGMP and cAMP to promote the water secretion and urine production via the activation of V-ATPase [25]. The With-No-Lysine kinases (WNKs) in human kidneys phosphorylate two related kinases, Ste20-related proline/alanine-rich kinase (SPAK) and oxidative stress response (OSR1) [32]. The SPAK and OSR1 further phosphorylate and active solute carrier family 12 (SLC12) transporters, including sodium-chloride (NCC), sodium-potassium-2-chloride 1 (NKCC1), and NKCC2 [33]. The WNK in the fruit fly regulates ions transport through activation of NKCC mediated by Fray in the PCs [34,35]. In mammals, the phosphorus hormone fibroblast growth factor 23 (FGF23) binds with FGF receptor 1 (FGFR1) and one klotho protein (KL) to regulate the Pi secretion. The homolog of FGF23 in the fruit flies is called Bnl, which acts on its receptor Btl and one klotho (KL)-like protein Kl on the basal membrane of the PCs to induce the expression of *MFS2*, thus increasing Pi uptake by the PCs [21]. Hormone Leukokinin (LK) acts on its receptor leukokinin receptor (LKR) located on the basal of the SCs to increase chloride ion conductivity [25].

3. The establishment of fruit fly kidney stones models

3.1. The establishment of CaOx stones model

The CaOx stones model in fruit fly was first established by Chen et al. (2011) via dietary treatment with lithogenic agents. They fed fruit fly with NaOx, EG, or HLP for two weeks and then observed the formation of the dose-dependent crystals in the MTs of the fruit flies treated with these lithogenic agents [18](Figure 2A). The NaOx administration supplies dietary oxalate, the excess oxalate binds with Ca^{2+} to form CaOx stones in MTs. The EG is metabolized sequentially to glycolaldehyde, glycolate, glyoxylate, and oxalate in the end *in vivo* (Figure 3A) [10]. The usage of EG administration is limited by the high toxicity of glycolate, which leads to chronic metabolic acidosis and significantly reduces the lifespan of fruit flies besides the stone formation [18,36]. The HLP is a precursor of oxalate. HLP is first converted to 4-hydroxy-2-oxoglutarate (HOG) in a three-step reaction Then the HOG is further converted to glyoxylate by HOG aldolase (HOGA). Finally, the glyoxylate is oxidized to the oxalate by lactate dehydrogenase (LDH) (Figure 2A) [37]. Chung et al. (2017) identified differentially expressed genes in the fruit fly fed with the NaOx or EG diets compared with the control group. Of these genes, 58 were expressed differently in the NaOx treated group and 20 genes were expressed differently in the EG treated group. No overlapping genes between the two treatment groups were detected, which was probably due to the different metabolic pathways of NaOx and EG. Several candidate genes were highlighted in the study, including *nervana 3*, *CG7912, excitatory amino acid transporter (Eaat 1)*, *crystallin* in the NaOx treatment group; *CG3036* and *CG5404* in the EG treatment group. Nervana 3 is the β subunit of Na^+/K^+ ATPase. *CG7912* encodes a protein predicted to be involved in the sulfate transmembrane transport. Eaat 1 mediates concomitant uptake of sodium and dicarboxylate. *Crystallin* functions as a calcium ion binding protein. In the EG treatment group, *CG3036* and *CG5404* were predicted as activators of the sodium-phosphate symporter and the sulfate transmembrane transporter [38].

A new CaOx stone model was developed by down-regulating the function of *dAGXT* without feeding any lithogenic agent to fruit flies by Yang et al. (2018) [19](Figure 2A). *AGXT* encodes Alanine-glyoxylate aminotransferase (AGT), which catalyzes the transamination between L-alanine and glyoxylate (Figure 3A). When AGT is reduced, the conversion of glyoxylate to alanine is decreased, and the accumulated glyoxylate is easily converted to oxalate by various dehydrogenases and oxidases like LDH, this results in the increased oxalate producing and CaOx crystal deposition in MTs [19,36].

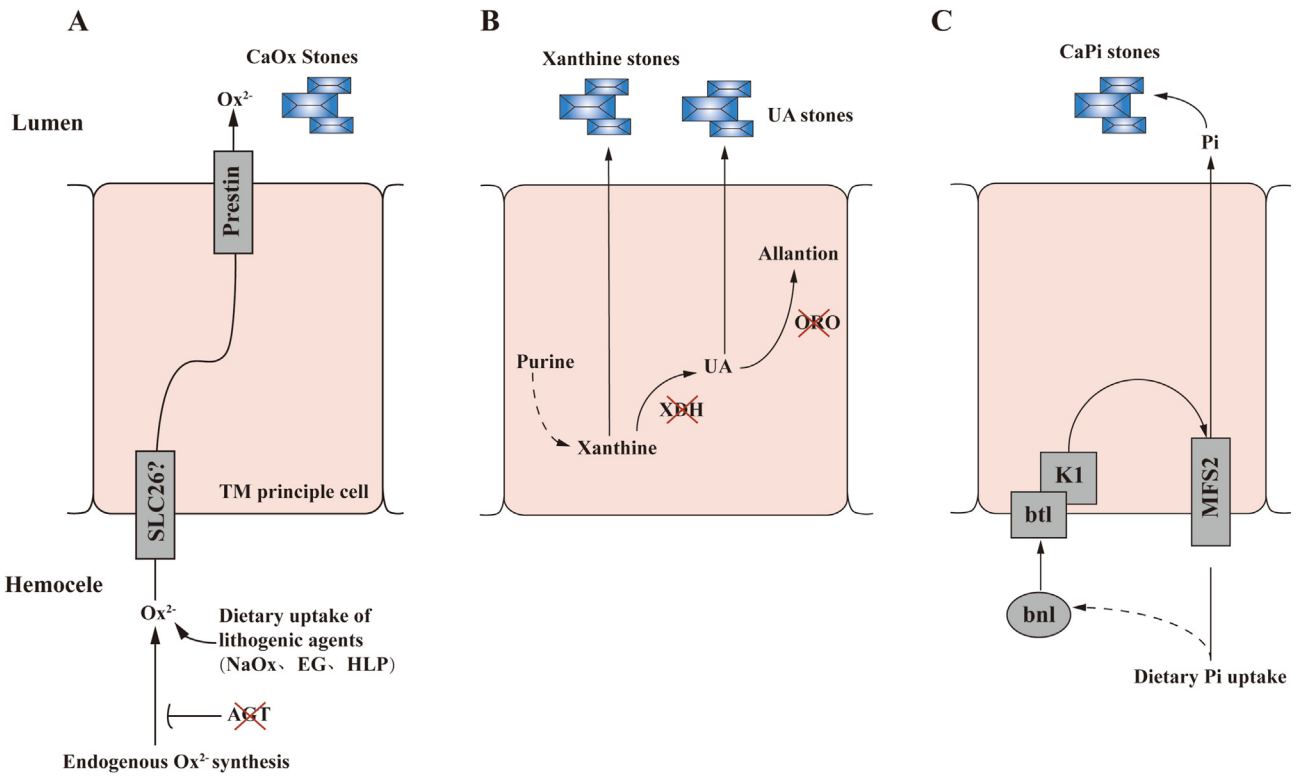

Figure 2. The major establishment principles of CaOx stone, xanthine stone, UA stone, and CaPi stone disease models. (A) Dietary and metabolize endogenous oxalate is transported to MT lumen through *Prestin* and another unknown SLC26 transporter. Overdose dietary uptake of oxalate ion (Ox^{2-}) or its metabolic precursors (HLP, EG) increases the concentration of Ox^{2-} in MT lumen and facilitates the CaOx crystalization. Genetically knockdown of the expression of AGT increases the endogenous oxalate synthesis, bringing similar results. (B) In the wild-type fruit fly, purine can be digested to allantoin as an end product. Knockdown XDH blocks purine metabolisms at xanthine, results in xanthine stones, and knockdown *Uro*, results in UA stones. (C) Overdose dietary phosphate (Pi) uptake up-regulates the expression of Pi transporter MFS2 via bnl/btl/MFS2 pathway, results in the increase of lumen Pi concentration, and CaPi stone formation.

3.2. Establishment of xanthine stones and UA stones

Mitchell and Glassman (1959) established a xanthine stone model in fruit fly MTs by mutating Xanthine dehydrogenase (Xdh), encoded by *rosy* [39](Figure 2B). Xdh converts both hypoxanthine to the xanthine and the xanthine to the uric acid (Figure 3B) [40]. Using this model, Chi et al. (2015) utilized RNAi system to silence *Xdh,* causing increased xanthine and hypoxanthine levels, and decreased uric acid levels in the fruit fly, which further resulted in the xanthine stone formation in the MTs. Moreover, the inhibition of Xdh by allopurinol led to a similar stone formation. This study also demonstrated the functional correlation between the levels of zinc and the xanthine stone formation in the urinary system. Zinc plays an important role in ectopic calcification. Zinc levels depend on the amount of yeast in the fly's diet. A high yeast diet (high protein diet) results in a high rate of stone formation due to excessive zinc uptake. Three Zinc transporters (ZnTs), ZnT35C, ZnT41F, and ZnT63C, are widely expressed in the MTs and implement the zinc secretion there. The silence of all these three transporters simultaneously significantly reduces the stone formation in the MTs by preventing the zinc secretion into the lumen of the tubule. Only ZnT35C is almost exclusively expressed in the MTs. ZnT35C inhibition alone also leads to the strongest reduction in the stone formation and the strongest stone rescue phenotype [41]. The zinc homeostasis in the MTs of the fruit fly is also mediated by Zip71B. Zip71B is localized in the basolateral plasma membrane, which is involved in zinc transport from the body into the MTs and acts upstream of the apically resident ZnT35C. MT-specific knockdown of *Zip71B* reduces the zinc accumulation in the tubules and mitigates the formation of tubule stones caused by the inhibition of *Xdh*. But the high body zinc levels decrease the survival of fruit flies in this situation [42].

Unlike humans, in which the functional urate oxidase (Uro) has been lost, the fruit fly has urate oxidase to convert uric acid into allantoin. Lang et al. (2019) established a fruit fly UA stone model by knocking down *uro*. *uro* knockdown plus high purine dietary increased UA levels and accumulation of UA stone in MTs of fruit fly and finally caused a shortened lifespan (Figure 2B). In this study, a correlation between the insulin/insulin-like growth factor signaling pathway (IIS) and the UA stone formation was also detected [20]. Hyperuricemia is a well-known complication of diabetes [43]. Moreover, the diabetic foot ulcer was newly found significantly associated with diabetic kidney disease and renal failure [44]. Recently, Dam et al. (2020) also discovered the high-sugar diet resulted in both elevated UA levels and acidification conditions in the fruit fly uric system, which promoted the UA stone formation in the MTs. In addition, a study on the formation of fruit fly UA stone model has shown that the interaction between the IIS pathway and the purines metabolism is conserved from the insects to the mammals [45].

The sex-determined region Y protein interaction protein-1 (Sip1), a homolog of the human Na^+/H^+ exchange factor (NHERF1), has a novel effect on renal UA stone formation. Sip1 and Na^+/H^+ exchanger isoform 2 (NHE2) co-localize to the apical membrane of tubule SCs, and Sip1 regulates NHE2 to regulate H^+ in the lumen, resulting in the lumen acidification and the UA stone formation [40].

Teashirt (Tsh) is a transcription factor required in the SCs differentiation and function establishment. It regulates the expression of *drip*, *ClC-a,* and *lkr* in SCs during the development, all of which are required for urine production. Compared with the wild type, the fluid secretion of the *tsh* knockdown tubules is significantly reduced, inducing the accumulation of the UA crystals in the lumen [25].

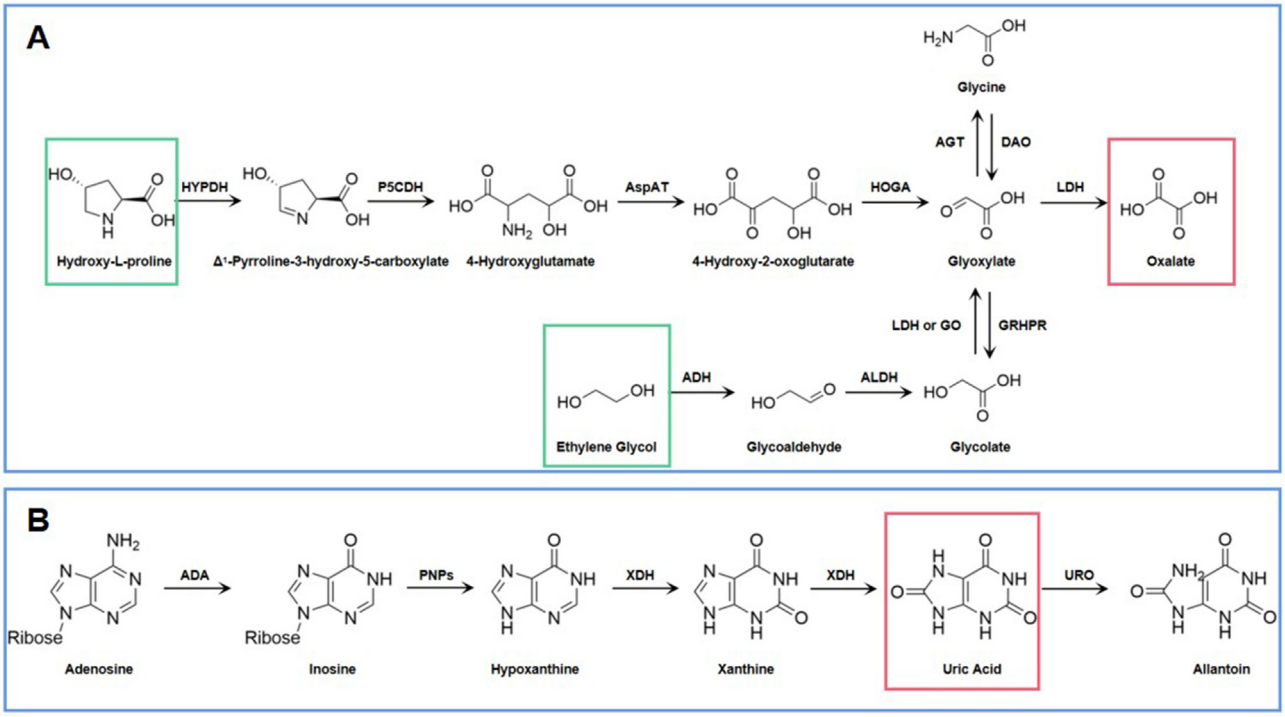

Figure 3. The biosynthesis pathways of (A) oxalate [36,37,63](B) uric acid [40]. Compounds in the green boxes are common lithogenic agents for CaOx kidney stone models. Abbreviations: hydroxyproline dehydrogenase (HYPDH), Δ1-pyrroline-5-carboxylate dehydrogenase (P5CDH), aspartate aminotransferase (AspAT), 4-hydroxy-2-oxoglutarate aldolase (HOGA), alanine-glyoxylate aminotransferase (AGT), D-amino acid oxidase (DAO), lactate dehydrogenase (LDH), alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), glycolate oxidase (GO), glyoxylate reductase/hydroxypyruvate reductase (GRHPR), adenosine deaminasepurine (ADA), nucleoside phosphorylase (PNPs), xanthine dehydrogenase (XDH), urate oxidase (URO).

3.3. Establishment of CaP stones

Rose et al. (2019) presented that the dietary Pi induces the expression of the fly type I sodium-pi co-transporter MFS2, resulting in the formation of CaP stones in the MTs and the lifespan shortening of fruit fly (Figure 2C). Knocking down of *MFS2* increased the Pi levels in the hemolymph and reduced the CaP stone formation in the MTs of fruit flies fed with a high Pi diet. Microinjection of FGF23 into the fruit fly leads to the MFS2 expression in the MTs and the reduction of the Pi levels in the hemolymph. Overexpression of *fly FGF branchless* (*bnl*), the FGF-like peptide, in the PCs increases the expression of MFS2, implicating bnl as a phosphaturic hormone in the fruit fly. Sevelamer is a polycation that regulates the serum phosphate to inhibit the CaP stone formation by binding to Pi and reducing its absorption from the diet. Phosphonoformic acid (PFA), an antiviral drug that blocks the Pi transporter, decreases the cell uptake of Pi and blocks the Pi excretion in the MTs to inhibit the CaP stone formation [21]. Table 1 listed the most important fruit fly models of kidney stone disease.

Table 1. Fruit fly models of kidney stone disease.

Stone type	Model	Driving force	Genes	Drugs	Reference
CaOx	NaOx supplement	Dietary Oxalate uptaking	*Nervana 3, CG7912, Eaat 1, crystallin*	Hydroquinone β- D-glucoside, K-HCA, HCA, *Garcinia cambogia*, BS168	Chen et al, 2011
	EG supplement	Dietary uptaking of Oralat precursor	*CG3036, CG5404*	*Salviae miltiorrhizae, Paeonia lactiflora, Carthami flos, Corydalis yanhusuo, Imperata cylindrica, Prunus armeniaca, Eclipta prostrata, Artemisia argyi, Plantago asiatica, Lonicera japonica, Polygoni cuspidati, Astragalus membranaceus, Wolfiporia cocos, Scutellaria baicalensis, Angelicae sinensis*	
	HLP supplement	Dietary uptaking of Oralat precursor			
	dAGXT (AGT) knockdown	Increasing endogenous Oxalate synthesis	*dAGXT*	N-Acetyl-L-hydroxy proline, Baclofen	Yang et al, 2018
xanthine	*Xdh* knockdown	Block the xanthine catabolism	*ZnT35C, ZnT41F, ZnT63C, Zip71B*		Mitchell and Glassman, 1959
UA	*Uro* knockdown	Block the UA catabolism	*Uro*		Lang et al, 2019
	Sip1 knockdown	Increasing lumen acidification	*Sip1*		Ghimire et al, 2019
	tsh knockdown	Block the SC differentiation	*Tsh, Drip, ClC-a, Lkr*		Denholm et al, 2013
CaP	sodium phosphate supplement	Dietary uptaking of CaP	*MFS2, bnl*	Phosphonoformic acid, Sevelamer	Rose et al, 2019

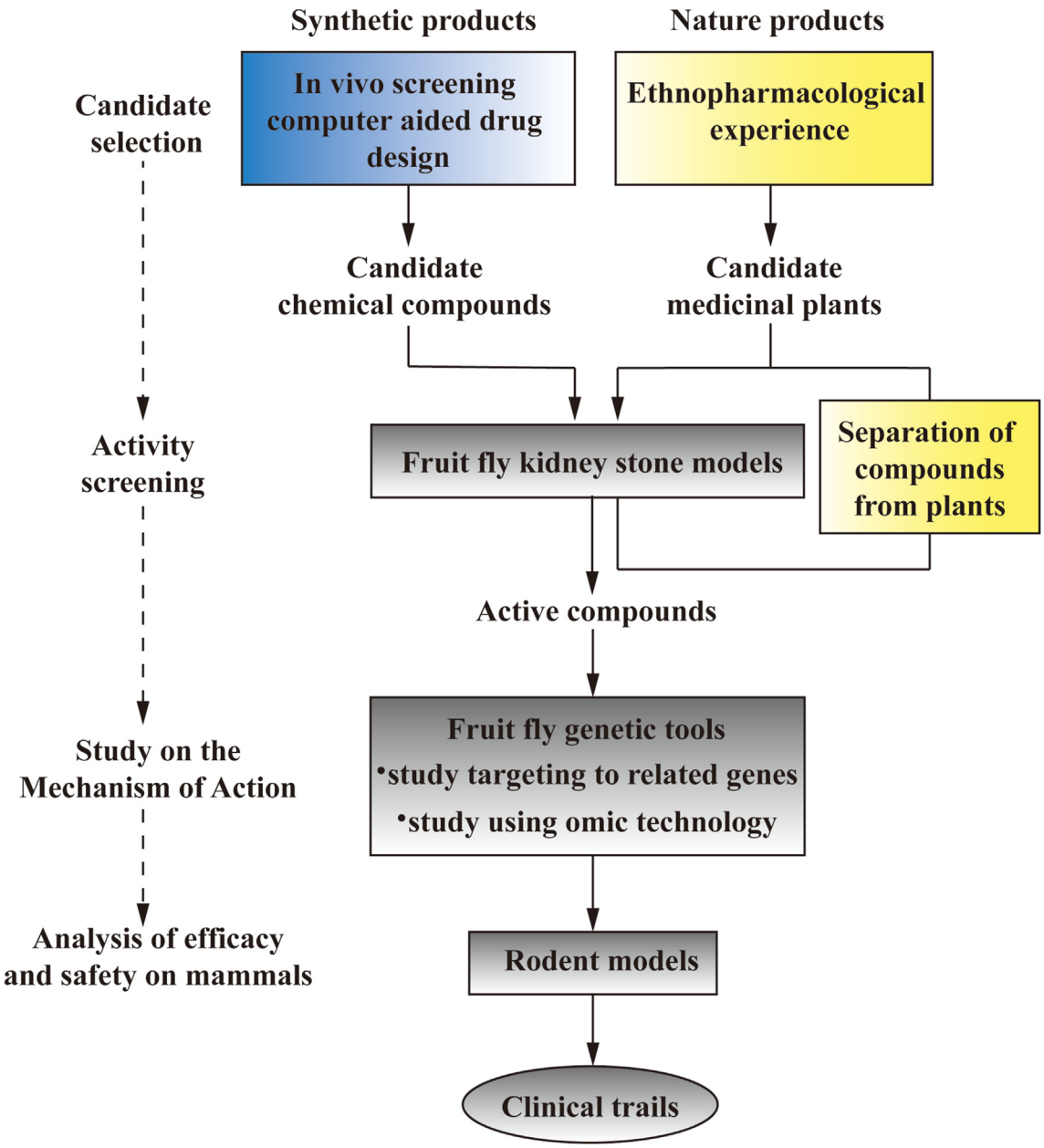

Figure 4. The workflow of using fruit fly kidney stone models in the novel drugs screening.

4. Application in drug development

Hydroquinone β-D-glucoside, a compound commonly known as arbutin was discovered to have antilithogenic effect first in the fruit fly kidney stone model induced by the NaOx administration. Using this model, arbutin was identified as a novel compound, which can bind to both free calcium ions and oxalate before the stone formation [46]. The citrate (CA) can complex calcium equivalent and inhibit CaOx monohydrate nucleation [47], and Potassium citrate (K-CA) has long been used to treat kidney stones [48]. *In vitro* studies show that hydroxycitrate (HCA) is a potential ramification of CA, which can dissolve the CaOx monohydrate crystal surface [49]. Using The fruit fly CaOx stones model, the crystal inhibition activities of hydroxy citric acid tripotassium (K-HCA) were the first exhibit *in vivo*. Compared with the K-CA, K-HCA had a stronger inhibitory effect to prevent the CaOx stone formation in the MTs of the NaOx treated fruit flies and maybe a better substitute [50]. HLP is an oxalate precursor. The HLP analogs N-Acetyl-L-hydroxy proline and 4-amino-3-(4-chlorophenyl) butyric acid (Baclofen) have been identified to inhibit the CaOx stone formation in *dAGXT* knockdown fruit fly at very low concentrations. These analogs reduce the conversion of glyoxylate to oxalate by inhibiting the activity of proline dehydrogenase [19]. The application of microorganisms in the treatment of CaOx kidney stones has also made new progress. Both Humans and fruit flies lack the enzymes to break down oxalate. But certain gut microbe can degrade the oxalate, making the intestinal microbiome has become a new focus for a novel treatment for kidney stone patients. *Lactobacillus* is the most commonly studied genus for this application [51,52]. However, they are not the only probiotics, that can degrade oxalate, *Bacillus subtilis* was also identified as an oxalate degrading bacterium *in vitro* since 2000 [53]. A recent *in vivo* study showed that *Bacillus subtilis* 168 (BS168) reduces CaOx stones in the MTs, increases the lifespan, and prevents the oxalate-induced microbiota dysbiosis of the fruit fly [54].

Plant-based traditional medicines play a worldwide important role in primary health care, especially in developing countries. The lack of systematic molecular studies limited the usage and exploitation of the potential values of traditional medicines. The fruit fly kidney stone models

provide an efficient, low-cost, and accurate way to study the efficacy and molecular mechanisms of traditional medicines against kidney stones. More than 80 traditional Chinese medicinal (TCM) plants were tested using the EG-induced CaOx stone model of the fruit fly, of which 15 plants were determined to have an antilithogenic ability. These 15 herbs are *Salviae miltiorrhizae*, *Paeonia lactiflora*, *Carthami flos*, *Corydalis yanhusuo*, *Imperata cylindrica*, *Prunus armeniaca*, *Eclipta prostrata*, *Artemisia argyi*, *Plantago asiatica*, *Lonicera japonica*, *Polygoni cuspidati*, *Astragalus membranaceus*, *Wolfiporia cocos*, *Scutellaria baicalensis* and *Angelicae sinensis* [55,56], have huge potentials for the future clinical application. For example, *Astragalus membranaceus* and *Salviae miltiorrhizae* are well-known TMC drugs for treating chronic kidney disease [57]. Their effects were confirmed in the clinic and with mammalian models [58, 59]. Further studies should use the fruit fly kidney stone models to identify the bioactive compounds present in such medicines and the effect mechanisms behind their efficacy. In another study, *Garcinia cambogia* extract was detected containing 60% hydroxycitric acid (HCA), which can directly dissolve CaOx. In the fruit fly CaOx model, both HCA and *Garcinia cambogia* play important roles in the prevention and treatment of CaOx stones in the MTs [6].

The fruit fly kidney stone model is a powerful tool for discovering and assaying novel agents for kidney stone controlling, including chemical compounds, natural compounds from medicinal plants, and probiotics. Certainly, fruit fly being an invertebrate model may not entirely reflect the human kidney. For example, although Baclofen was found to have an inhibition effect on CaOx stone formation in fruit fly MT, it was also reported to have nephrotoxicity in humans [60]. Hence, the results from fruit fly models still need to be complemented with studies on mammalian models to test the safety and effectiveness before the clinical trials.

5. Concluding remarks and prospects

Kidney stone disease is a worldwide problem, the pathogenesis is not clear and its effective treatment is scarce. The Malpighian tubules of the fruit fly are functionally analog to the human kidney. The metabolism and genetic regulation of the urinary systems are conserved between mammals and insects. The Fruit fly plays an important role in the mechanism research and drug developments for various diseases, such as cancers, diabetes, and neurodegenerative diseases. Several FDA-approved drugs have been first discovered using fruit fly models [61,62].

Several kidney stone models have been established by the dietary and genetic approaches newly over the past decade. To date, these stone models have been applied in drug development and identified various chemical compounds, medicinal plants, and microorganisms, which have therapeutic and preventive effects. These researches suggest fruit fly also offers a promising platform for studying human kidney stone diseases, especially to be used as an efficient, rapid screening platform for novel drugs, which is required for the management of kidney stones. Here we draw a workflow chart to reveal a general idea of how to use fruit fly kidney stone models in the novel drugs screening (Figure 4). Both candidate synthetic products base on *in vitro* screening [60] or bioinformatics prediction and plants from traditional medicinal experience can be analyzed by fruit fly models. The active compounds of the validated active medicinal plants will be further found out due to screening with the same model. The target and mechanisms of action of the active compounds can be further studied with the fruit fly model. The powerful genetic tools and well-annotated genome of fruit flies will facilitate the study of certain related target genes (Table 1) and non-target studies using omic technology. The efficacy and safety of the active compounds should confirm on mammalian models again before the clinical trials. However, the prestudy on fruit fly will reduce the huge amount of time and cost for this purpose. In general, the fruit fly is a powerful tool for the study of the pathogenesis of kidney stone diseases and will strongly contribute to the development of novel antilithogenic treatments in the future.

Declarations

Author contribution statement

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