RESEARCH ARTICLE

In-vivo Evaluation of Nephroprotective Activity of Naringenin against ADPKD and MDCK-derived Cysts

T. Naga Varalakshmi[1*], V. Chitra[2]

[1]*Department of Pharmacology, Pulla Reddy Institute of Pharmacy, Hyderabad, Telangana, India.*
[2]*Department of Pharmacology, SRM College of Pharmacy, SRMIST, Chennai, Tamil Nadu, India.*

Received: 18th April, 2023; Revised: 07th July, 2023; Accepted: 25th August, 2023; Available Online: 25th September, 2023

ABSTRACT

Aim: Determining the *in-vivo* assessment of Naringenin's nephroprotective efficacy against Autosomal dominant polycystic kidney disease (ADPKD) and Madin-Darby canine kidney (MDCK) derived cysts.

Method: Our research on the molecular mechanism of naringenin, a flavonoid present in plants and berries that has been shown to limit cell growth and protect against cancer in *in-vitro* and animal models, was conducted using dictyostelium, a simple, controllable biomedical model. Cultured MDCK cells were used to generate differentiated tubule cells, and these findings were extrapolated to a human kidney model employing these cells.

Results: While naringenin inhibited growth in dictyostelium, it had no effect on development. In a random-gene-knockout screen, a TRPP2 (polycystin-2) knockout mutant was discovered to be resistant to naringenin's effects on growth and random-cell movement. Changes in the divalent transient receptor cause polycystic kidney disease type 2 potential cation channel TRPP2. We found that the growth of cysts and MDCK cells might be inhibited by naringenin. Partial resistance to naringenin was achieved in this model by lowering TRPP2 levels via siRNA, as evidenced by the presence of larger cysts following treatment with 3 and 10 M naringenin compared to controls. Naringenin had no effect on chloride secretion.

Conclusion: Naringenin's influence on cell proliferation is mediated by TRPP2 in both dictyostelium and mammalian kidney cells, despite their vast evolutionary distance from one another (polycystin-2). Naringenin will be the subject of more research as a possible new therapeutic treatment for ADPKD.

Keywords: ADPKD, Cyst growth, Dictyostelium, MDCK, Naringenin

International Journal of Pharmaceutical Quality Assurance (2023); DOI: 10.25258/ijpqa.14.3.54

How to cite this article: Varalakshmi TN, Chitra V. *In-vivo* Evaluation of Nephroprotective Activity of Naringenin against ADPKD and MDCK-derived Cysts. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):776-785.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

A prevalent hereditary condition known as "polycystic kidney disease (PKD)", cysts in the kidneys enlarge and multiply, which ultimately cause chronic renal failure.[1] "Autosomal dominant polycystic kidney disease (ADPKD)" represents the vast majority of cases of PKD. Polycystic kidney disease is caused by a deficiency in either the "polycystin-1 (PC1)" or "polycystin-2 (PC2)" proteins, both of which are encoded by mutations in the PKD1 and PKD2 genes. Reduced intracellular Ca2+ and a rise in cAMP are the results of PC1 or PC2 mutations.[2] When intracellular cAMP levels increase, The epithelial lining of a cyst expands, causing fluid to flow into the cyst cavity.[3] Cyst epithelial cell proliferation is aberrant due to alterations in Wnt, B-Raf/MEK/ERK, and mTOR signaling pathways.[4] Several pieces of data show chlorine transporter in the "Cystic fibrosis transmembrane conductance regulator (CFTR)" is essential for keeping ADPKD cysts from filling with fluid and electrolytes.[5] Small-molecule CFTR inhibitors have been shown to reduce cyst development in both *in-vitro* and in vivo models of PKD. Some examples are the CFTR-172 thiazolidinone inhibitor and the CFTR inhibitors made from phenyl-derivatized glycine hydrazide. Additionally, in Pkd1 deletion mice, CFTR inhibitors slowed the growth of cysts and preserved renal function.[6] It has been established that nuclear receptors like PPAR and LXR can inhibit CFTR-mediated chloride secretion and reduce the rate at which renal cysts form.[7] The evidence is in favor of blocking CFTR as a possible treatment for PKD.

Agents that reduce cell proliferation have been demonstrated to halt cyst progression in PKD animal models.[8] The treatment of ADPKD has been linked to multiple targets, include mTOR, the cell cycle, and receptors for vasopressin and calcium.[9] A significant finding is that metformin, an anti-diabetic medication, activates the 50 "AMP-activated protein kinase

**Author for Correspondence: varalakshmi.54t@gmail.com*

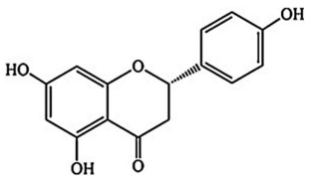

Figure 1: Naringenin (4′,5,7-trihydroxyflavanone) Structure

(AMPK)", that inhibits cell growth and release of fluid via the CFTR pathway.[10] The glucose analog 2-deoxy-D-glucose (2DG) also reversed AMPK deactivation and slowed PKD progression in a mouse model.[11] As a result, AMPK activators may one day serve as therapeutic agents for the creation of drugs for the treatment of PKD.

Most plant-derived compounds are readily absorbed and digested by the body, and some are considered healthy natural organic components.[12] People now have a more comprehensive understanding of phytochemical components because of modern science and technology advancements. Numerous illnesses, including cancer, metabolic disorders, and neurodegeneration, have been treated with thousands of plant-derived chemicals.[13] Numerous substances with plant origins have also been researched for their potential advantages in slowing the evolution of ADPKD.

In recent years, several drugs, including tolvaptan, rapamycin, and somatostatin, have shown promise in halting the progression of ADPKD. These developments have been noteworthy. Tolvaptan was the first medicine the FDA approved specifically for treating ADPKD. It has the ability to reduce the rise of TKV and the water loss, and possible liver damage despite a reduction in estimated glomerular filtration rate (eGFR) that other therapeutic measures are required.[14] In addition, the mTOR inhibitor rapamycin and its analogs failed to demonstrate sufficient therapeutic results in clinical investigations.[15]

The broad family of plant polyphenols known as flavonoids, which includes the flavonone subclass, includes "Naringenin (4′,5,7-trihydroxyflavanone)" (Figure 1). Naringenin is mostly eaten as Naringin, which is also its glycoside, and is present in grapefruit juice in rather high concentrations (30 mg naringin 100 mL^{-1}). Naringenin is bioavailable at modest quantities; for instance, following absorption, Blood levels of the aglycone were measured at 0.7 to 14.8 M after a dosage of 139 to 265 mg of naringenin.[16] Low micromolar quantities of naringenin glucuronides and sulfates are seen following phase 2 metabolism.[17] Like many flavonoids, Naringenin inhibits tumor cell proliferation by inducing dependent on p53 for apoptosis.[18] In colon cancer-prone mice, naringenin inhibits tumor growth and spread as well as tissue invasion.[19] As part of its chemopreventive actions, naringenin may activate particular enzymes called cytochrome P450 and phase 2 to help the body get rid of carcinogens.[20]

In this model, we tried to figure out how naringenin works at the molecular level because earlier research[21] revealed that naringenin stopped *Dictyostelium* cells from growing and dividing. A library of these mutants was tested to find the genes that make *dictyostelium* REMI mutants resistant to naringenin. Using the right mammalian system, researchers then looked into how the found gene The substance mediated naringenin's effects on cell growth. A nonselective Ca^{2+}-permeable cation channel known as polycystin-2 (TRPP2) has been connected to "Autosomal dominant polycystic kidney disease",[22] has been discovered by our data as a new option for how naringenin affects how cells work. In this condition, fluid secretion into the cysts and a malfunction in proliferation cause kidney cysts to form.[23] About 85% of instances of ADPKD are caused by "TRPP1 (polycystic kidney disease-1)" gene and protein mutations, while 15% are due to TRPP2 (PKD2) protein and gene mutations.[24] We thus investigated the role of TRPP2 in naringenin's As a follow-up to our first findings in *Dictyostelium*, we examined the influence effects on cyst development and "Madin-Darby canine kidney (MDCK)" cell proliferation. We discovered that in both systems, nar ingenin's growth-inhibitory effects were mediated by TRPP2 (polycystin-2).

The maximum oral bioavailability of naringenin is 5.81% because it dissolves slowly in water (approximately 46 g/mL) and has a low affinity for water. Glucuronide intermediate metabolites are quickly formed during the first-pass metabolism in the liver. Its bioavailability in plasma is severely restricted. After taking the traditional Chinese medicine Zhi Zhu Wan by mouth, Sun *et al*. found that naringenin's half-life is approximately 4.69 hours specifically, AUC = 40,617.9 ng/mL and maximum concentration (C_{max}) = 2,910.6 ng/mL[25,26] C_{max} = 2019.51 +/ 760.81 ng/mL and AUC (0-infinity) = 9434.51 +/ 2860.51 ng/mL were observed after 3.5 hours of oral administration of 135 mg naringenin. In spite of the ease with which it is absorbed, the scientists hypothesized that due to the significant first-pass metabolism that takes place in the colon, naringenin's bioavailability remained low. A significant amount of naringenin was discovered in the blood and urine of the animal after drinking either naringin or grapefruit juice.[27] Numerous potent naringenin formulations have been developed in recent years by employing a wide variety of procedures, some of which include sophisticated inclusion processes.[28-30]

Naringenin is not highly hazardous, with a fatal dose of only 5000 mg/kg.[31] Naringenin's primary mode of action is the inhibition of CYP1A2 and CYP3A4 in human cytochrome P450. The antagonistic effects of naringenin have been successfully found to work on every subtype of opioid receptor. It has been shown to benefit human health, with several research studies confirming its hepatoprotective,[32] qualities that protect against mutation, carcinogenesis, oxidative stress, diabetes, and atherosclerosis. It has been suggested that a few hundred mg of flavonoids are the minimum amount of flavonoids that should be consumed daily.[33] Because of its ability to reduce inflammation and protect cells from oxidative damage, it has been recommended as a potential treatment for a variety of illnesses related to oxidative stress.[34]

Accordingly, naringin boosts glutathione levels and antioxidant enzyme activity to prevent damage to the kidneys, reduces lipid peroxidation, and inhibits inflammatory cytokine production.[35] Molecular analyses have shown that naringin inhibits autophagy, suppresses apoptosis, and activates Nrf-2. In an acute kidney injury model, naringin modulated renal tissue microRNA-10a, protecting the kidney.[36] Cardiotoxicity and nephrotoxicity from paclitaxel in older male Wistar rats: effects of naringin and naringenin as inhibitors.[37] As a dietary supplement, naringenin has been proposed for use in the management of diabetes and other conditions associated with oxidative stress, which raises the possibility that it might have a similar impact on the management of diabetes in people. Lead, a heavy metal, and naringenin, a flavanone, were studied *in-vivo*.[38] The fourth group of rats was the control. In order to perform the CA enzyme assay on rat erythrocytes, the other three groups received a mixture of lead and naringenin or either of those substances alone. In comparison other communities, the control group's enzyme activity was the greatest. Naringenin caused the most inhibition and lead the least. As a result, naringenin is an effective inhibitor of the CA enzyme.

MATERIALS AND METHOD

Assays of *Dictyostelium* Growth

Wild-type (Ax2) *Dictyostelium discoideum* cells were isolated from axenic media at the midpoint of the log phase of growth. (4 106). After 24 hours, 1 106 cells•mL of cells were resuspended in axenic medium containing either 100 M naringenin or DMSO.

Library Screening using *Dictyostelium* Insertional Mutagenesis

(Hiroyuki Adachi, Takeshi Hasebe, Keisuke Yoshiunga, 1994) pBBC plasmids were made by modifying the pBSR1 plasmid in various ways include 60mer DNA barcodes, to mutate wild-type dictyostelium cells (Ax4). The enzyme pairs were the most efficient restriction enzyme combinations for plasmid linearization/electroporation in REMI. In 24-well culture plates, transformants were created clonally and frozen in 10% DMSO for later recovery. On a bacterial growth plate, cells remaining after plating were identified, cultured for two days, and then harvested to create pools. (24 mutants per pool). A total of 30 significant pools of 672 to 768 mutants were created by combining a number of these 24 pool combinations (28–32 pools). The enrichment tests were conducted for each pool using 25 big pools, 106 mutant cells were grown in 10 mL of HL-5 with 100 g/mL streptomycin and 100 μ/mL penicillin naringenin in two 10 cm Petri dishes.[39] When the cell density reached 2 x 106 cells/mL at the end of the third day, the previous culture media containing 1-mL was removed, and 9 mL of new media with naringenin were combined. For 21 days, the identical process was carried out again. Purifying cells from each growing dish for their genomic DNA and cloning plasmid insertion locations in accordance with previously published methods.[40] In order to confirm that the mutant was resistant to naringenin-induced growth suppression and to continue studying it, the insertion site of a clonal strain came from a frozen supply that had been stored in a 24-well freezer.

Recapitulation of *Dictyostelium* pkd2

Utilizing the techniques previously described, knockout constructs were produced (Pakes *et al*., 2012). In a nutshell, polymerase chain reaction (PCR) amplification of using genomic DNA, the flanking 5′ and 3′ segments of the Dictyostelium pkd2 gene were analyzed from wild-type organisms. CTGATATTGCCT CATTCCATGGCTTCG and TTGGTGATTGTGGGGTACCAG TAC were used to amplify the 3′ terminal fragment, while AAGGGATCCAATACCTTGAAATTAA TAATCCATC and TTAACTGCANGCTGATGCTGTC were used to amplify the 5′ terminal targeted segment. Both the 5′ (500 bp) and 3′ (744 bp) The gene was cloned from PCR fragments into the plPBLP expression vector in the opposite direction of the blasticidin resistance cassette using the restriction sites BamHI/PstI and NcoI/KpnI. Since we linearized the knockout cassette, elec troporation yielded wild-type cells. Isolating transformants was a breeze with the help of blasticidin-containing nutritional medium (10 g•mL). Diagnostic PCR products were generated from target gene genomic DNA and knockout vector sequences, and they were used to test individual clones for homologous integration. Following the identification of three separate isolates, Raoultella planticola was clonally plated with the isolates, and isogenic colonies were employed in further investigations.

Assay for the Random Migration of *Dictyostelium* Cysts

After 48 hours in Axenic medium, Cells of wild-type and pkd2 were extracted, cleaned, and resuspended at 1.7 106 cells/mL before being exposed to 30 nM cAMP for six minutes at a time while being shaken at 120 rpm. The purpose of these experiments was to determine how naringenin affects Dictyostelium random cell migration.[41] Following one hour of shaking in 8-well glass coverslips with Naringenin 200 M or 0.7% DMSO (solvent control), cells were randomly moved and photographed every 15 seconds for 5 minutes to document this process. A minimum of three independent tests were carried out for each cell and condition, with an average of about 10 cells being quantified in each experiment. Quimp 11b, an ImageJ plugin, was used to examine random cell movement.[42] In MATLAB, we observed how the presence or absence of naringenin affected the speed, direction, range, and total number of protrusions for each cell line. The effects of naringenin on random cell motion were analyzed, including how it impacted the cells' ability to move in circles, the number of protrusions they made, and the distance they traveled. We compared the differences between the groups using Tukey's post hoc test and one-way ANOVA.

MDCK Cell Culture

A 10% FBS, 40 g mL^{-1} gentamycin, 100 units mL^{-1} penicillin, and 1040 g mL^{-1} streptomycin DMEM medium was used to cultivate MDCK cells. This was done at 37°C in an environment

containing 5% carbon dioxide and a humidified atmosphere. In each and every test of cytotoxicity and cyst formation, cells with fewer than 18 passage numbers were used.

MDCK Cell Viability Assays

Assaying with sulforhodamine B (SRB) allowed us to find out if naringenin had an antiproliferative impact on MDCK cells when it was mixed with DMSO (up to 0.1% DMSO by volume). Over the course of 48 hours, naringenin (1-100 M) was added to the 96-well plate in which 10,000 cells had been seeded and allowed to adhere for 24 hours. After taking out the medium, after incubating the plates at room temperature for 10 minutes, we poured 100 L of 0.4% (w/v) SRB in 1% (v/v) acetic acid into each hole. After the SRB solution was taken away, the cells were washed with 1% acetic acid five times and then dried at room temperature in the air. To remove SRB from adherent cells, plates were shaken horizontally in 10 mM tris base (unbuffered) at room temperature for at least 10 minutes. Using an ELx808 Absorbance microplate reader, the absorbance was determined to be 550 nm. The gold standard for this study was actinomycin-D. (5 M). As was previously disclosed, measurements of 510 nm were used to test for neutral red absorbance.[43,44] EC50 values were computed using GraphPad Prism's four-parameter non-linear regression.

MDCK Cyst Culture and Mmeasurement

We adhered to a recognized protocol for cyst growth investigations. In 24-well plates, MDCK cells were grown, to put it briefly. 800 cells per well of a cell suspension were added, along with a chilly 0.4 mL of PureCol by Nutacon BV ice-cold collagen, 10 mM HEPES, and 27 mM $NaHCO_3$. Gentamycin, penicil, and streptomycin were also added. Collagen-containing cell-filled plates were approximately 2 hours in an incubator at 37°C, with humidity, and 5% carbon dioxide. Before reincubating the plates, Forskolin (10 M) and fetal bovine serum (10% FBS) were added to DMEM (1.5 mL/well). Fresh DMEM media supplemented with forskolin was added to the cells until the end of day 12. (10 M). Within three days of plating, the cells developed into cysts, they were then 100X magnified and photographed using an inverted microscope. At the end of day 6, 50 cysts were chosen, and every cyst with a diameter larger than 50 m grids made from microscope slides were placed in each compartment. The MDCK cells that had been transfected underwent the same procedure. On days 6, 8, and 10, DMEM (1.5 mL per well) with 10 M forskolin was mixed with 1, 3, 10, 30, 60, and 100 M maximum concentrations of 0.1% DMSO for naringenin, 10 M metformin, vehicle control (0.1% DMSO), and vehicle to see if there was any effect on cyst formation. On days 6, 8, 10, and 12, pictures were taken of the cysts in question. Image J was used to figure out how big the cyst was (mm^2).

MDCK Monolayer Electrophysiological Studies

A seed density of 2 x 105 cells per cm^2 of Snapwell permeable supports was used for the plating of MDCK cells. In culture, cells organized into monolayers in an atmosphere at 5% CO_2 and 37°C. After 12 to 13 days, monolayers were employed in experiments, additionally, every two days, new media was introduced. The Krebs-Henselheit (KH) buffer was applied to both sides of the monolayers set up in using chambers. In mM, the following chemicals make up the KH buffer. To keep the pH at 7.45, 95% O_2 and 5% CO_2 are used to bubble the water. Buffer tanks with heated water jackets allowed for experiments to be conducted at 37°C. The Bio-Pac MP100 A/D converter, Acknowledge 3.8.2 software, and DVC-1000 voltage-clamp amplifier were used to record the short-circuit current. (ISC). MDCK monolayers exhibited a 0 mV transepithelial potential difference (Vt). To calculate the transepithelial resistance, the change in current following brief voltage changes between 0 and 1 mV (2 seconds) was used. The spontaneous Vt of fixed MDCK monolayers was 4.7 1.3 mV (n = 12) an average ISC of 1.0 0.3 A•cm2, a transepithelial resistance of 2152 308 cm2, and an ISC of 1.0 0.3 A•cm^2 on average.

siRNA Transfection of TRPP2

Up to 80% of the MDCK cells were confluent. Before transfection, cells were cultured in reduced serum media called Opti-MEM overnight for transfection experiments. After that, cells were transfected for 24 hours either siRNA with a random sequence utilizing lipofectamine 2000, a lipid-based transfection agent, either human or canine TRPP2 siRNA was transfected at a final dose of 20 nM (per well). (Invitrogen). Cells were given 12 hours of antibiotic-free recovery time in DMEM. Cells were re-transfected with the same method the next day. The transfection efficiency of this double transfection method was between 60 and 70%. Cells were cultured for another 24 to 48 hours after transfection before doing Western blotting, cyst culture, or a proliferation experiment. Confocal microscopy was used to analyze the subcellular localization and stability of siRNA in transfected cells grown in chambered glass slides. According to the Concise Guide to Pharmacology published by the British Pharmacological Society (BJP), TRPP2 (polycystin-2) has been designated as a molecular target.[45]

Analysis via Western Blotting

Cell lysates were extracted from both control and transfected cells using RIPA (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, and 1 mM EDTA). Using 4 to 12% bis-tris acrylamide gels, protein samples (50 g) were separated before being transferred to nitrocellulose membranes and incubated overnight at 4°C with mouse monoclonal anti-actin antibody or anti-TRPP2 antibody in PBS. Following an hour of incubation at room temperature with IR Dye anti-goat/mouse secondary antiserum, the blots were washed three times with PBS/0.01% TWEEN 20. Infrared imaging methods were used to quantify immunostained proteins.

RESULTS

***Dictyostelium* Growth is Inhibited by Naringenin**

We first set up conditions for screening a *Dictystelium* REMI mutant collection to figure out how naringenin works at the

molecular level. Wild-type *Dictyostelium* was observed with an EC50 between 50 and 100 M, naringenin stopped after 48–96 hours of growth in shaking cultures (Figure 2A). However, *Dictyostelium* grew normally at up to 200 M naringenin concentrations on nitrocellulose screens, where the cells unite to form fruiting bodies. We were able to isolate cells resistant to naringenin by exposing a pool of REMI mutants to 200 M naringenin in a shaking culture for 21 days. Under these circumstances, naringenin-resistant cells' sequence analyses identified 26 mutants with a broken open reading frame, which may regulate naringenin's effect on cell proliferation. The pkd2 gene was deleted, which would have produced the terminated TRPP2 protein (DDB_G0272999). The molecular mass of this protein was also identical to the human protein, coming in at 82.2 kDa (715 aa) (variant 1; 87.0 kDa; 758 aa). It shared 26% sequence identity with the human protein and was 49% comparable to it, and both had the same number of putative transmembrane domains (Figure 2).

Naringenin's Action on *Dictyostelium* is Mediated by TRPP2

By producing the pkd2 Inactivated mutant allele of a gene in parental cells, the effects of nariningenin on pkd2 cells and wild-type cells were studied (Figure 4). First results from these two cell lines showed that wild-type cell proliferation was greatly reduced by 100 M naringenin at 48 and 72 hours (P 0.001) (Figure 5), while the growth of pkd2 mutant cells was slower than that of wild-type cells when naringenin was not present but did not change significantly when at both times, 100 M of naringenin was added. To get a better idea of how big this resistant phenotype was, and because we had already shown how different dietary chemicals affect how *Dictyostelium* cells act (Robery *et al*., 2011), we also looked into how naringenin might affect how cells move and how they look. In these tests, cells were grown under controlled conditions for 5 hours. We observed and recorded their random cellular motion after 60 minutes of treatment with 200 M naringenin. You can see that naringenin stops wild-type cells from moving and makes them round up (Figure 2B, D). The pkd2 mutant, on the other hand, didn't change much when naringenin was added, based on how circular it was (Figure 2B), how many pseudopods it had (Figure 2C), or how the cells moved (Figure 3). (Figure 2D). Based on these results, TRPP2 is in charge of how naringenin changes *Dictyostelium* moves.

MDCK Cell and Cyst Growth is Suppressed by Naringenin

Given that human TRPP2 protein mutations are linked to the growth of stones form in the kidneys of people with "polycystic kidney disease (PKD)" (Chapin & Caplan, 2010), Next, we looked into whether naringenin inhibits cell growth in mammals by binding to TRPP2. Cyst development in the MDCK canine kidney due to its similarity to the human PKD1 cell line, expression of TRPP2 (Schlatterer & Malchow, 1993) (X. Li et al., 2005). Using two different assays to measure cell growth, Using EC50 values between 28.51 M, naringenin

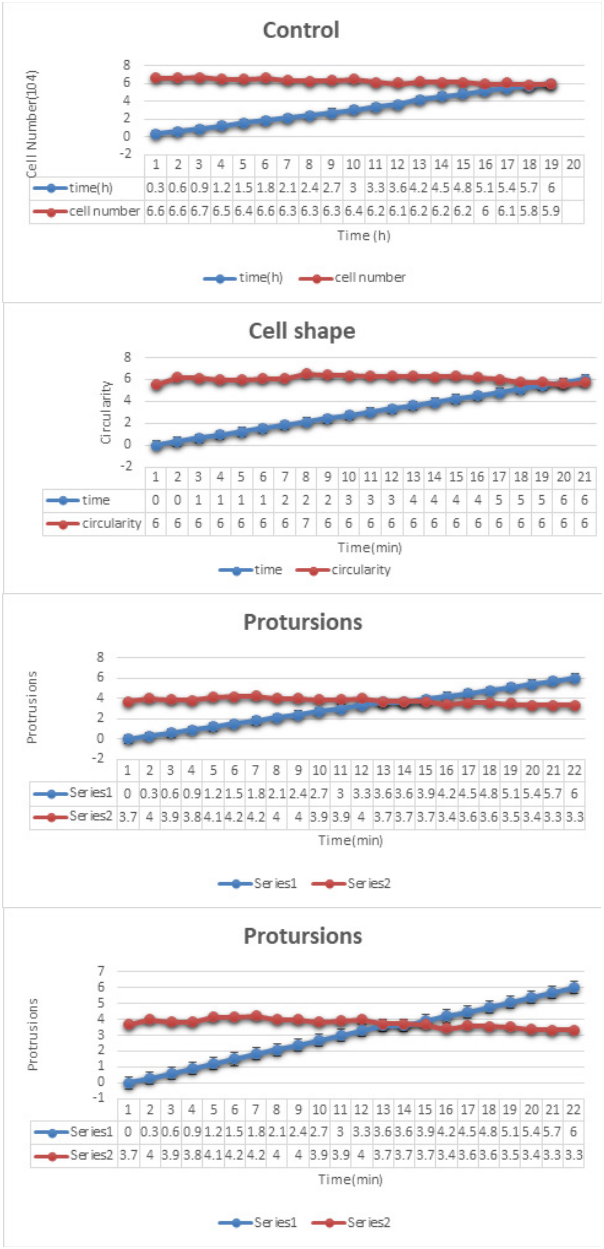

Figure 2: Naringenin's inhibitory effects on the growth and cellular activity of *Dictyostelium* were controlled by the TRPP2 protein. (A) In a concentration-dependent manner, naringenin suppresses dictyostelium development in liquid culture. (B) After being exposed to naringenin, When compared to wild-type cells treated to vehicle, these cells were substantially more circular (blue circles). (C) Cells of the wild type treated with naringenin (blue circles) pseudopod development was drastically reduced weighed against pkd2 mutant cells and vehicle controls, and (D) Treatment with naringenin significantly reduced motility in wild-type cells.

inhibited MDCK cell growth in this system during a time period of 24 to 48 hours (Figure 3). (neutral red assay and SRB).

Two different assays, neutral red and SRB protein, were used to determine cell viability. For three different tests, we give both the average and the variation in the average. To find EC50 values, we performed a non-linear regression analysis. Half-effective doses (EC50) were calculated to be 28.1 mM

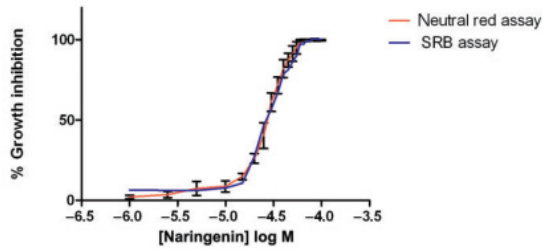

Figure 3: Proliferative, non-polarized MDCK cells were inhibited by naringenin after 48 hours

(neutral red test) and 291 mM (SRB assay). R-values greater than 0.98 were observed.

We then looked at how naringenin affected the development number of tumors in this cell line, course of six days after a 12 day treatment to see if there's a way to use this method for making cysts. Twelve days after induction, six days of exposure to 100 M naringenin completely inhibited growth; at this dose, no cysts persisted. Another study revealed that the AMP-dependent kinase activator metformin (10 M) also suppressed cyst development (Takiar et al., 2011).

Polycystin-2 (TRPP2) Knockdown Shields MDCK Cysts and Cells from Naringenin

We employed the siRNA method to reduce TRPP2 expression in MDCK cells because development of those cells and cysts was inhibited by naringenin. Two proteins (90 and 130 kDa) were discovered by western blotting of MDCK cell extracts from separated cell culture, confirming the presence of TRPP2 (Liang et al., 2008). After letting the cells heal for up to 48 h and transfecting them siRNA targeting TRPP2 RNA or a control RNA. Western blot analysis could be used to measure how much MDCK protein had been lost. Both TRPP2 siRNA and TRPP2 + scrambled siRNA were used at concentrations of 10 nmol. (TRPP2 siRNA only), the number of TRPP2 proteins was reduced in a dose-dependent manner using this approach. After 24 hours, protein levels dropped to 56.5, 398.2% after 48 hours, and 32.2% after 12 days when 20 nmol of TRPP2 siRNA was given to the cells. (Figure 4).

After 48 hours of exposure to increasing doses of naringenin, transfection of MDCK cells with either a control siRNA or TRPP2 siRNA was followed by an assessment of their ability to proliferate. When comparing cells transfected with scrambled siRNA to those that weren't transfected, we found that the TRPP2 siRNA transfected cells were more resistant to the growth-inhibiting effects of naringenin. (Figure 6). Figure 3 displays an increase from 28.1 M to 65 M in the EC50 value for naringenin. The EC50 for growth suppression was not affected by transfection with randomly modified siRNA. (30 M). At a point when TRPP2 protein was 39% of control levels, naringenin's EC50 value had to be more than twice that to suppress cell proliferation (48 hours) (Figure 5).

For 6 days, cysts were grown in MDCK cells without transfection or in pre-treatment TRPP2 siRNA-transfected cells with naringenin (1–100 M). (until day 12). Evidence that decreased functional TRPP2 levels promote cyst

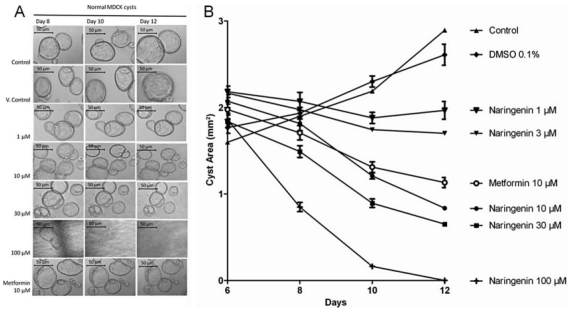

Figure 4 : Naringenin inhibits cyst development. Normal cysts (A) Normal cysts captured in microscopic detail. (B) Relationship between cyst size and therapy

production and expansion can be seen in the increased size of transfected cysts with knocked-down TRPP2 compared to their untransfected counterparts. Transfected cysts at day 6 showed increased resistance to naringenin's suppression at 10 M and 30 M compared to control cysts. (Figure 7C) (at day 12). After 12 days, cysts were no longer visible when treated with 100 M naringenin. Further supporting the selectivity of TRPP2 siRNA therapy was the observation that Transfected cyst growth was stifled by 10 M metformin without affecting the expansion of normal cysts. (Figure 7B).

Naringenin had No Effect on Chloride Secretion in MDCK Monolayers

Super-physiological quantities of naringenin (EC50 > 330 M) have been found to decrease chloride secretion (in the rat colon), however, other studies have found that it stimulates more chloride to be expelled. Cyst growth can be controlled by decreasing cAMP-dependent chloride secretion, for example. Then, we looked into how narin genin affected ISC (forskolin-induced chloride secretion) (Figure 8). Forskolin (at 20 mM) was added to the monolayers after they were pretreated Using either 30 microMolar (M) naringenin or its transporter, 0.1% dimethyl sulfoxide (DMSO). When forskolin was administered for ten minutes, there was no difference in ISC between monolayers treated with vehicle (2.0 0.4 cm2,

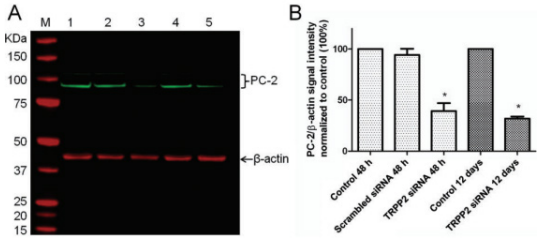

Figure 5: MDCK cells with knocked-down TRPP2 (PC-2). Cells transfected with TRPP2 for 12 days (lanes 4-7). (lanes 4–5). In lanes 1 and 2, cells were not transfected with siRNA for 48 hours; lanes 3 and 4 were transfected with TRPP2 siRNA for 48 hours; lanes 5 and 6, respectively, were transfected with TRPP2 siRNA for 12 days. (12 days). Molecular signature; M. There was a 30 g/lane concentration of sample loading. (B) Fluorescence intensity of TRPP2/-actin was normalized to that of nontransfected cells. Estimated mean deviation, n = 3–5. In comparison to a day-matched control group, *p > 0.05

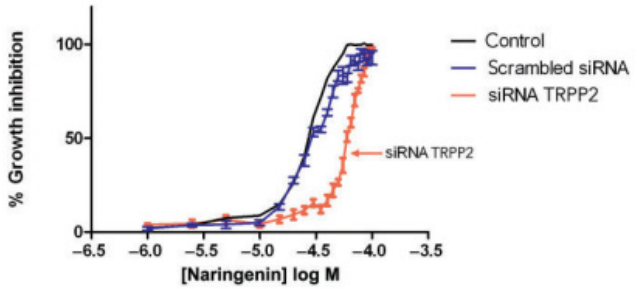

Figure 6: After 48 hours, naringenin's inhibitory effects on TRPP2 siRNA-transfected MDCK cell growth diminished. Scrambled siRNA transfection followed by naringenin therapy had no effect on cell growth.

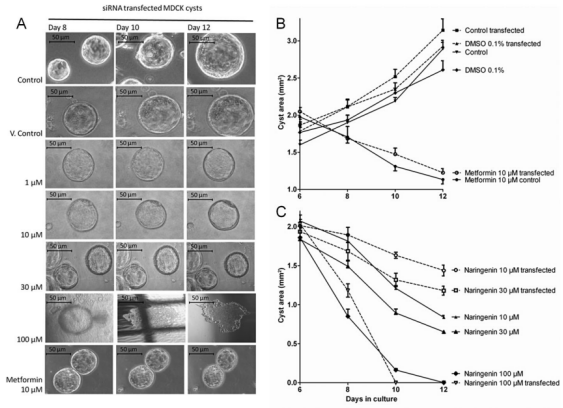

Figure 7: The growth-inhibiting effects of naringenin can be avoided by transfecting cysts with siRNA targeting TRPP2. (A) Photographs of transfected cysts treated for 12 days (B) Cyst size as a function of whether the cysts were exposed to medium (control) (C) before and after naringenin treatment, as well as in transfected cysts.

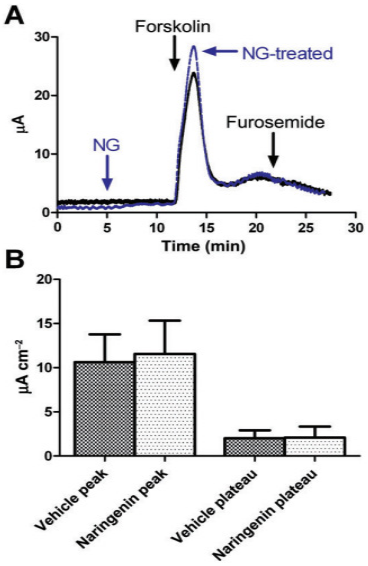

Figure 8: Alteration of cAMP-dependent chloride secretion by naringenin. (A) Typical short-circuit current traces demonstrating the effects of naringenin (B) Summary results (mean SEM, n = 6)

n = 6) and naringenin (2.1 0.5 cm^2, n = 6; p 0.05, one-way ANOVA). Furosemide was given after the forskolin response had already started to decrease. Furosemide is an inhibitor of basolateral chloride absorption. ISC were largely unaffected, showing that forskolin's effect on stimulating chloride secretion was transient.

DISCUSSION AND CONCLUSIONS

Dictyostelium growth is stifled by naringenin by way of TRPP2

We present the first evidence that TRPP2 is involved in naringenin's previously reported reduction in renal tubular cell growth and cyst formation. *Dictyostelium* served as a model organism for pharmacogenetic studies, allowing for the determination of this effect and the mechanism by which it operates. Compared to the previously reported value of 20 mM, our results demonstrated that naringenin considerably inhibited *Dictyostelium* growth at concentrations between 50 and 100 mm.[46] After 60 minutes of treatment, naringenin reduced cell behavior (shape, creation of pseudopods, and random cell movement). It was found that the naringenin-inhibitory action was provided pkd2 mutants failed to experience the naringenin-dependent change in cellular phenotype this resistance was most likely caused via means of TRPP2 protein production. The growth of *Dictyostelium* was discovered to be stifled by the flavonoid apigenin. Naringenin may have structural specificity via a TRPP2-dependent impact, as it appeared to have an effect when quercitin and chrysin did not.

Although the method by which TRPP2 controls the behavior of *dictyostelium* cells is unknown, calcium signaling has been implicated in *Dictyostelium*.[47] Our results demonstrate that naringenin induces changes to the cytoskeletal structure and prevents cell migration behavior were supported by the findings of these studies, which demonstrated that there is an instantaneous drop in calcium levels outside of cells reduced cell motility and led to detachment from the supporting substrate. The breakdown of the cell's internal structure led to this. Additionally, BAPTA loading hindered cell mobility and pseudopod emission by chelating intracellular calcium.[48] In the future, studies might also take into account TRPP1, which is the name of a possible protein that plays a role in pkd1 in *Dictyostelium* (DDB_G0289409).

Naringenin Blocks the Development of Kidney Cells via TRPP2

Naringenin stopped the renal MDCK cell and cyst growth at EC50 values comparable to those of Dictyostelium (50 M). Inhibition of this protein by siRNA showed that TRPP2 was responsible for regulating the growth-inhibitory impact of naringenin on MDCK cells, and discovered that TRPP2 expression was present in these mammalian cells. TRPP2 has been studied extensively in kidney tubule cells because to its multifaceted role in mammalian cells. Polycystin-2 (TRPP2), The endoplasmic reticulum and the major cilium both include this ion channel, which belongs to the transient

receptor potential (TRP) family.[42] The major cilium kinks in response to shear stress, which triggers TRPP2 and allows Ca^{2+} to enter the cell. Ca^{2+} inflow stimulates TRPP2 on the ER, which causes Ca^{2+} release and increases intracellular Ca^{2+} concentration, promoting cell development. Ca^{2+} promotes PDE and maintains a low level of cAMP, it prevents further growth of cysts by blocking the Ras/Raf/MEK/ERK pathway. PKD1 and PKD2's equivalent mutations disrupt TRPP1 or TRPP2, cause flow-sensitive PKD. Cyst proliferation results from decreased Ca2+ influx, increased cAMP, and activation of Ras/Raf/MEK/ERK pathway.

We propose that activation of TRPP2 by naringenin might facilitate Ca^{2+} influx and inhibit cellular growth. Numerous channels. Several ion channels, The naringenin has been demonstrated to modulate a variety of cellular processes, including the "big conductivity (BK)" Inducible kinase activity in vascular myocytes.[48] It is intriguing that naringenin can inhibit development by attaching to TRPP2 and turning it on.

The TRPP1/TRPP2 complex has been shown to affect growth regulation via multiple signaling pathways, including Ca^{2+} and cAMP, mTOR, STAT1/3, Proliferation, apoptosis, and differentiation are all controlled by G proteins, and the -catenin/Wnt pathway is responsible for cell division.[49] The interaction between TRPP2 and the proliferation-promoting transcription factor Id2 is particularly intriguing. Mutations in TRPP2 create a scenario where Id2 can enter the nucleus and mute growth-restricting genes, leading to ADPKD. Hypothesized to inhibit cell proliferation by a mechanism similar to phospho-ERK-driven eIF2 phosphorylation, TRPP2 promotes eIF2 phosphorylation.[50] It is important to learn more about these channels' role(s) in naringenin's activity.

Remember that neither metformin nor naringenin lost any of their ability to suppress cyst development after being transfected. Metformin upregulates AMPK, a kinase that works with mTOR to control growth.[42] Essential for Cl secretion, apical "CFTR (cystic fibrosis transmembrane conductance regulator)" channels are also downregulated via the "AMP-activated protein kinase (AMPK)" pathway.[45] Metformin shows considerable promise as a possible therapy option for ADPKD due to its ability to reduce cystogenesis and chloride secretion.

Cl secretion is not Inhibited byNringenin

Genestein and apigenin, among other flavonoids, control the amount of chloride secreted across the epithelium via the CFTR.[43] Consistent with a prior observation, forskolin caused a temporary increase of ISC in MDCK cells. Neither ISC nor the response to forskolin were affected by Naringenin.[46] These findings suggest that a decrease in cAMP-dependent chloride secretion was not the cause of the decrease in cyst development.

There are no therapeutic treatments for PKD yet authorized.[41] An additional, more risky choice involves direct channel blockade or metformin-induced CFTR inhibition (X. Li et al., 2005; Takiar et al., 2011); metformin- or Inhibiting B-Raf is a key process along the MAPK/ERK pathway. We can make assumptions based on our findings about naringenin's possible role in the therapy of ADPKD. Naringenin probably won't benefit ADPKD patients because 15% of them no longer have TRPP2 function. In those with the majority of ADPKD cases, naringenin may activate TRPP2 and prevent cyst development if TRPP2 is present but TRPP1 is dysfunctional. This would present a novel treatment approach for the management of ADPKD. More investigation is now required to determine whether naringenin can effectively treat ADPKD.

REFERENCES

1. Abdul-Majeed S, Nauli SM. Calcium-mediated mechanisms of cystic expansion. Biochim Biophys Acta Mol Basis Dis. 2011;1812(10):1281–1290. https://doi.org/10.1016/j.bbadis.2010.09.016.
2. Alam MA, Subhan N, Rahman MM, Uddin SJ, Reza HM, Sarker SD. Effect of citrus flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action. Adv Nutr. 2014;5(4):404–417. https://doi.org/10.3945/an.113.005603.
3. Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M, Peters JA, Harmar AJ. The concise guide to PHARMACOLOGY 2013/14: Transporters. Br J Pharmacol. 2013;170(8):1706–1796. https://doi.org/10.1111/bph.12450.
4. Amini N, Maleki M, Badavi M. Nephroprotective activity of naringin against chemical-induced toxicity and renal ischemia/reperfusion injury: A review. Avicenna J Phytomed. 2022;12(4):1–14. https://doi.org/10.22038/AJP.2022.19620.
5. Armendáriz-Barragán B, Zafar N, Badri W, Galindo-Rodríguez SA, Kabbaj D, Fessi H, Elaissari A. Plant extracts: from encapsulation to application. Expert Opin Drug Deliv. 2016;13(8):1165–1175. https://doi.org/10.1080/17425247.2016.1182487.
6. Bastos AP, Onuchic LF. Molecular and cellular pathogenesis of autosomal dominant polycystic kidney disease. Braz J Med Biol Res. 2011;44(7):606–617. https://doi.org/10.1590/s0100-879x2011007500068.
7. Belibi FA, Reif G, Wallace DP, Yamaguchi T, Olsen L, Li H, Helmkamp GM, Grantham JJ. Cyclic AMP promotes growth and secretion in human polycystic kidney epithelial cells. Kidney Int. 2004;66(3):964–973. https://doi.org/10.1111/j.1523-1755.2004.00843.x.
8. Blair HA. Tolvaptan: A Review in Autosomal Dominant Polycystic Kidney Disease. Drugs. 2019;79(3):303–313. https://doi.org/10.1007/s40265-019-1056-1.
9. Calvet JP. Strategies to inhibit cyst formation in ADPKD. Clin J Am Soc Nephrol. 2008;3(4):1205–1211. https://doi.org/10.2215/CJN.05651207.
10. Capuano I, Buonanno P, Riccio E, Rizzo M, Pisani A. Tolvaptan vs. somatostatin in the treatment of ADPKD: A review of the literature. Clin Nephrol. 2022;97(3):131–140. https://doi.org/10.5414/CN110510.
11. Chang MY, Ong ACM. New treatments for autosomal dominant polycystic kidney disease. Br J Clin Pharmacol. 2013;76(4):524–535. https://doi.org/10.1111/bcp.12136.
12. Chapin HC, Caplan MJ. The cell biology of polycystic kidney disease. J Cell Biol. 2010;191(4):701–710. https://doi.org/10.1083/jcb.201006173.
13. Chiaravalli M, Rowe I, Mannella V, Quilici G, Canu T, Bianchi V, Gurgone A, Antunes S, D'Adamo P, Esposito A, Musco G, Boletta A. 2-Deoxy-D-glucose ameliorates PKD progression. J Am Soc Nephrol. 2016;27(7):1958–1969. https://doi.org/10.1681/

ASN.2015030231.
14. Erlund I, Meririnne E, Alfthan G, Aro A. Human nutrition and metabolism: Plasma kinetics and urinary excretion of the flavanones naringenin and hesperetin in humans after ingestion of orange juice and grapefruit juice. J Nutr. 2001;131(2):235–241. https://doi.org/10.1093/jn/131.2.235.
15. Fache S, Dalous J, Engelund M, Hansen C, Chamaraux F, Fourcade B, Satre M, Devreotes P, Bruckert F. Calcium mobilization stimulates Dictyostelium discoideum shear-flow-induced cell motility. J Cell Sci. 2005;118(15):3445–3457. https://doi.org/10.1242/jcs.02461.
16. Harris PC, Torres VE. Polycystic kidney disease. Annu Rev Med. 2009;60:321–337. https://doi.org/10.1146/annurev.med.60.101707.125712.
17. Hiroyuki Adachi, Takeshi Hasebe, Keisuke Yoshiunga, T. ohta and K. S. Isolation of Dictyostelium discoideum cytokinesis mutants by restriction enzyme mediated integration of the blasticidin s restiance marker. Biomedical and Biophysical Research Communications. 1994;204(4):1808–1814.
18. Hollman PCH, Katan MB. Dietary flavonoids: Intake, health effects and bioavailability. Food Chem Toxicol. 1999;37(9–10):937–942. https://doi.org/10.1016/S0278-6915(99)00079-4.
19. Kanaze FI, Bounartzi MI, Georgarakis M, Niopas I. Pharmacokinetics of the citrus flavanone aglycones hesperetin and naringenin after single oral administration in human subjects. Eur J Clin Nutr. 2007;61(4):472–477. https://doi.org/10.1038/sj.ejcn.1602543.
20. Katavic PL, Lamb K, Navarro H, Prisinzano TE. Flavonoids as opioid receptor ligands: Identification and preliminary structure-activity relationships. J Nat Prod. 2007;70(8):1278–1282. https://doi.org/10.1021/np070194x.
21. Khaled SS, Soliman HA, Abdel-Gabbar M, Ahmed NA, Attia KAHA, Mahran HA, El-Nahass ES, Ahmed OM. The Preventive Effects of Naringin and Naringenin against Paclitaxel-Induced Nephrotoxicity and Cardiotoxicity in Male Wistar Rats. Evid Based Complement Alternat Med. 2022;2022. https://doi.org/10.1155/2022/8739815.
22. Kuspa A. Restriction enzyme-mediated integration (REMI) mutagenesis. Methods Mol Biol. 2006;346:201–209. https://doi.org/10.1385/1-59745-144-4:201.
23. Kuzu M, Özkaya A, Şahin Z, Dağ Ü, Çomakli V, Demirdağ R. Naringenin ve Kurşunun Sıçan Eritrosit Karbonik Anhidraz Enzimi Üzerine In Vivo Etkileri. Turk J Pharm Sci. 2017;14(1):9–12. https://doi.org/10.4274/tjps.13008.
24. Leonardi T, Vanamala J, Taddeo SS, Davidson LA, Murphy ME, Patil BS, Wang N, Carroll RJ, Chapkin RS, Lupton JR, Turner ND. Apigenin and naringenin suppress colon carcinogenesis through the aberrant crypt stage in azoxymethane-treated rats. Exp Biol Med (Maywood). 2010;235(6):710–717. https://doi.org/10.1258/ebm.2010.009359.
25. Li H, Findlay IA, Sheppard DN. The relationship between cell proliferation, Cl- secretion, and renal cyst growth: A study using CFTR inhibitors. Kidney Int. 2004;66(5):1926–1938. https://doi.org/10.1111/j.1523-1755.2004.00967.x.
26. Li X, Luo Y, Starremans PGG, McNamara CA, Pei Y, Zhou J. Polycystin-1 and polycystin-2 regulate the cell cycle through the helix-loop-helix inhibitor Id2. Nat Cell Biol. 2005;7(12):1102–1112. https://doi.org/10.1038/ncb1326.
27. Liang G, Yang J, Wang Z, Li Q, Tang Y, Chen XZ. Polycystin-2 down-regulates cell proliferation via promoting PERK-dependent phosphorylation of eIF2α. Hum Mol Genet. 2008;17(20):3254–3262. https://doi.org/10.1093/hmg/ddn221.
28. Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr. 2005;81(1 Suppl):230S–242S. https://doi.org/10.1093/ajcn/81.1.230S.
29. Meiyanto E, Hermawan A, Anindyajati. Natural products for cancer-targeted therapy: Citrus flavonoids as potent chemopreventive agents. Asian Pac J Cancer Prev. 2012;13(2):427–436. https://doi.org/10.7314/APJCP.2012.13.2.427.
30. Misty R, Martinez R, Ali H, Steimle PA. Naringenin is a novel inhibitor of Dictyostelium cell proliferation and cell migration. Biochem Biophys Res Commun. 2006;345(1):516–522. https://doi.org/10.1016/j.bbrc.2006.04.047.
31. Moon YJ, Wang X, Morris ME. Dietary flavonoids: Effects on xenobiotic and carcinogen metabolism. Toxicol In Vitro. 2006;20(2):187–210. https://doi.org/10.1016/j.tiv.2005.06.048.
32. Ortiz-Andrade RR, Sánchez-Salgado JC, Navarrete-Vázquez G, Webster SP, Binnie M, García-Jiménez S, León-Rivera I, Cigarroa-Vázquez P, Villalobos-Molina R, Estrada-Soto S. Antidiabetic and toxicological evaluations of naringenin in normoglycaemic and NIDDM rat models and its implications on extra-pancreatic glucose regulation. Diabetes Obes Metab. 2008;10(11):1097–1104. https://doi.org/10.1111/j.1463-1326.2008.00869.x.
33. Pakes NK, Veltman DM, Rivero F, Nasir J, Insall R, Williams RSB. Investigating the effect of emetic compounds on chemotaxis in Dictyostelium identifies a non-sentient model for bitter and hot tastant research. PLoS ONE. 2011;6(9). https://doi.org/10.1371/journal.pone.0024439.
34. Raksaseri P, Chatsudthipong V, Muanprasat C, Soodvilai S. Activation of liver X receptors reduces CFTR-mediated Cl-transport in kidney collecting duct cells. Am J Physiol Renal Physiol. 2013. https://doi.org/10.1152/ajprenal.00579.2012.
35. Ramakrishna Y, Goda H, Baliga MS, Munshi AK. Decreasing cariogenic bacteria with a natural, alternative prevention therapy utilizing phytochemistry (plant extracts). J Clin Pediatr Dent. 2011;36(1):55–64. https://doi.org/10.17796/jcpd.36.1.f485870h90174311.
36. Renugadevi J, Prabu SM. Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. Exp Toxicol Pathol. 2010;62(2):171–181. https://doi.org/10.1016/j.etp.2009.03.010.
37. Repetto G, del Peso A, Zurita JL. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. Nat Protoc. 2008;3(7):1125–1131. https://doi.org/10.1038/nprot.2008.75.
38. Rivoira MA, Rodriguez V, Talamoni G, de Talamoni NT. New perspectives in the pharmacological potential of naringin in medicine. Curr Med Chem. 2021;28(10):1987-2007.
39. Robery S, Mukanowa J, Percie du Sert N, Andrews PLR, Williams RSB. Investigating the effect of emetic compounds on chemotaxis in Dictyostelium identifies a non-sentient model for bitter and hot tastant research. PLoS ONE. 2011;6(9). https://doi.org/10.1371/journal.pone.0024439.
40. Saponara S, Testai L, Iozzi D, Martinelli A, Martelli A, Chericoni S, Sgaragli G, Fusi F, Calderone V. (+/-)-naringenin as large conductance Ca2+-activated K+ (BKCa) channel opener in vascular smooth muscle cells. Br J Pharmacol. 2006;149(8):1013–1021. https://doi.org/10.1038/sj.bjp.0706951.
41. Schlatterer C, Malchow D. Intracellular Guanosine-5'-O-(3-thiotriphosphate) Blocks Chemotactic Motility of Dictyostelium

discoideum Amoebae. 1993;307.
42. Simmons BYNL. BYNL SIMMONS From the Department of Physiological Sciences, The Medical School, University of Newcastle upon Tyne,.NE2 4HH (Received 16 February 1990). 459–472.
43. Sirovina D, Orsolic N, Gregorovic G, Koncic MZ. Naringenin ameliorates pathological changes in liver and kidney of diabetic mice: A preliminary study. Arh Hig Rada Toksikol. 2016;67(1):19–24. https://doi.org/10.1515/aiht-2016-67-2708.
44. Sun H, Dong T, Zhang A, Yang J, Yan G, Sakurai T, Wu X, Han Y, Wang X. Pharmacokinetics of hesperetin and naringenin in the Zhi Zhu Wan, a traditional chinese medicinal formulae, and its pharmacodynamics study. Phytother Res. 2013;27(9):1345–1351. https://doi.org/10.1002/ptr.4867.
45. Sussman M. Cultivation and Synchronous Morphogenesis of Dictyostelium under Controlled Experimental Conditions. 1991;28(C):9–29. https://doi.org/10.1016/S0091-679X(08)61635-0.
46. Takiar V, Nishio S, Seo-Mayer P, King JD, Li H, Zhang L, Karihaloo A, Hallows KR, Somlo S, Caplan MJ. Activating AMP-activated protein kinase (AMPK) slows renal cystogenesis. Proc Natl Acad Sci U S A. 2011;108(6):2462–2467. https://doi.org/10.1073/pnas.1011498108.
47. Torres VE, Chapman AB, Devuyst O, Gansevoort RT, Grantham JJ, Higashihara E, Perrone RD, Krasa HB, Ouyang J, Czerwiec FS. Tolvaptan in patients with autosomal dominant polycystic kidney disease. N Engl J Med. 2012;367(25):2407–2418. https://doi.org/10.1056/nejmoa1205511.
48. Tyson RANL. From the Department of Physiological Sciences, The Medical School, University of Newcastle upon Tyne,.NE2 4HH (Received 16 February 1990). 459–472.
49. Vassilev PM, Kanazirska M, Ye C ping, Brown EM, Guo L, Chen XZ, Segal Y, Peng JB, Basora N, Babakhanlou H, Cruger G, Hediger MA, Zhou J. Polycystin-2 is a novel cation channel implicated in defective intracellular Ca2+ homeostasis in polycystic kidney disease. Biochem Biophys Res Commun. 2001;282(1):341–350. https://doi.org/10.1006/bbrc.2001.4554.
50. Yang B, Sonawane ND, Zhao D, Somlo S, Verkman AS. Small-molecule CFTR inhibitors slow cyst growth in polycystic kidney disease. J Am Soc Nephrol. 2008;19(7):1300–1310. https://doi.org/10.1681/ASN.2007070828.