5-2024

Evaluation Of Wogonin And Chrysin In Alleviating Mycophenolate-Induced Diarrhea And Its Pharmacokinetics Profile

Abasifreke Sunday Benson

EVALUATION OF WOGONIN AND CHRYSIN IN ALLEVIATING MYCOPHENOLATE-INDUCED DIARRHEA AND ITS PHARMACOKINETICS PROFILE

THESIS

Presented in Partial Fulfilment of the Requirements for

the Master of Science Degree in the Graduate school

of Texas Southern University

By

Abasifreke Sunday Benson

Texas Southern University

2024

Approved by

Dr. Song Gao
Chairperson, Thesis Committee

Dr. Mahesh Vanjani
Dean, Graduate School

Approved by

Dr. Song Gao	3-27-24
Chairperson, Thesis Committee	Date
Dr. Huan Xie	3-27-24
Committee Member	Date
Dr. Yun Zhang	3-27-24
Committee Member	Date
Dr. Mario Hollomon	3-27-24
Committee Member	Date

EVALUATION OF WOGONIN AND CHRYSIN IN ALLEVIATING MYCOPHENOLATE-INDUCED DIARRHEA AND ITS PHARMACOKINETICS PROFILE

By

Abasifreke Benson

Texas Southern University, 2024

Dr. Song Gao, Advisor

Medication-induced diarrhea is a common adverse effect experienced by patients taking immunosuppressant medications used in Organ transplants. However, Mycophenolate mofetil (MMF) is the first-line drug to prevent organ transplant rejection. (Van Gelder & Hesselink, 2015) . MMF's mechanism of action involves inhibition of inosine-5'-monophosphate dehydrogenase. It is used for the treatment and management of a variety of autoimmune diseases as well as to prevent organ rejection in patients with bone marrow and solid organ transplants; however, over 50% of its adverse effects have been reported to be diarrhea. (Arslan et al., 2007; Farooqi et al., 2020). However, few options exist for managing the unwanted effects of MMF-induced diarrhea, which often leads to a subtherapeutic dose, as seen in alternatives such as Azathioprine (Sekmek et al., 2021) or using a low dose of MMF or withdrawal of an MMF which on the long-term may cause a high incidence of organ rejection, graft failure, and eventual patient mortality (Park et al., 2019).

MMF can affect the upper and lower GI tract, which often increases diarrhea intensity if left untreated (Parfitt et al., 2008a). Nevertheless, the area of alleviating MMF-induced diarrhea is grossly understudied; hence, one does not fully understand the precise mechanism underlying GI toxicity. However, enterohepatic recycling after glucuronidation has been implicated.

The labile acyl glucuronide (acylMPAG) and the inactive phenolic glucuronide (MPAG) are produced by glucuronidation. Although the main process of MPA detoxification in vivo is the creation of MPAG, research has attempted to connect acyl glucuronide synthesis with adverse drug reactions (ADRs) like MMF-induced diarrhea(Fukushima et al., 2021). For drugs like MMF, it is postulated that glucuronidation in the GI tract is insufficient, and this might result in excessive MPA and acyl MPAG accumulation in the lower GI segments to cause local toxicity, which manifests as diarrhea. In this study, we hypothesized that when we hydrolyze the active moiety of MMF causing MMF-induced diarrhea, that is, prevent MPAG from undergoing enterohepatic recycling to MPA or eventually AcMPAG, using the flavonoids Wogonin and Chrysin. We can successfully alleviate MMF-induced diarrhea and would also evaluate the dose-effect and eventual survival rate.

Following an already established diarrhea model, the female Wister Han used were divided into five groups; the first group was administered 70mg/kg/day MMF-only (Control), the other was given 50mg/kg/day of Wogonin (Treatment) and Chrysin (Treatment) separately. The next batch was also dosed with 100mg/kg/day of Wogonin and Chrysin separately. The non-control group (treatment group) received oral gavage of wogonin and chrysin separately at 50 mg/kg per day for two days before co-administering

with MMF as pretreatment. From day 3, MMF was administered orally to female Wistar Han rats at 70 mg/kg daily for seven consecutive days as an oral gavage. Subsequently, the indicators of illness activity (body weight, diarrhea score, and survival analysis) were observed. Plasma blood samples were taken on day 9, the seventh day of dosing with MMF, to monitor the PK profile of the various samples. Also, on day 10, GI tissues were collected before diarrhea to quantify tissue drug concentrations of MMF and MPA, MPAG, and AcylMPAG using LC-MS/MS.

Wogonin in both groups, orally administered with 100mg/kg/day and 50mg/kg/day of wogonin separately alongside MMF 70mg/kg/day in each batch, alleviated Mycophenolate Mofetil-induced diarrhea damage, which was indicated by reduced weight loss compared to control and diarrhea score, thereby preventing possible mucositis in the small intestine as well as the colon.

However, the chrysin batch was dosed orally with 100mg/kg/day and 50mg/kg group differently along with MMF 70mg/kg/day grade 3 and 4 diarrhea. The control group, which was administered only with MMF at 70mg/kg/day, had severe diarrhea and resulted in a nosedived survival rate following Survival analysis.

Nevertheless, after determining that wogonin and chrysin majorly influenced the disease activity index and survival index in all the MMF-diarrhea rats, we went further to check the Pharmacokinetic Profile of the five batches of the rat's plasma and tissues. Blood samples were collected at 0.25-hour, 0.50-hour, 1.00-hour, 2.00-hours, 4.00-hours, 6.00-hours, 8.00-hours, and 24.00 hours and used in the plasma preparation for quantitation. Subsequently, tissues were collected from the liver, colon, and ileum and processed and quantified using LC-MS.

In conclusion, a wogonin batch of animals survived throughout the experiment without any significant deaths, which showed that regardless of dose, Wogonin alleviated MMF-induced diarrhea and enhanced the rats' survival. These conclusions confirm our earlier postulation that flavonoids Like Wogonin and Chrysin can alleviate diarrhea in rats that have MMF-induced diarrhea, possibly by inhibiting the formation of toxic metabolites of MMF like MPA and AcylMPAG, thereby enhancing intestinal glucuronidation without significantly affecting the active metabolites MPA, which is in tandem with other reported in-vitro studies. Also, Wogonin has an enhanced local bioavailability in the GI Tract.

This insight could be useful in the management of Mycophenolate mofetil-induced diarrhea by mitigating the need to carry out further study on wogonin as an alternative to toxic chemicals in the management of organ MMF-induced diarrhea since it belongs to the naturally occurring flavonoids family, which are considered safe.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

LIST OF ABBREVIATIONS

%	Percentage
<	Less Than
µG	Microgram
µL	Microlitre
µM	Micromolar
µm	Micron
CE	Carboxylesterase
FDA	US Food and Drug Administration
LCMS	Liquid Chromatography Mass Spectrometry
MG	Milligram
mL	Milliliter
MMF	Mycophenolate mofetil
MPAG	Mycophenolate Acid Glucuronide
MPA	Mycophenolic Acid
UGT	UDP Glucuronosyltransferase
UGT1A1	Uridine Diphosphoglucuronate-Glucuronosyltransferase 1A1
US	United States
EHR	Enterohepatic recycling

VITA

2013.....B. Pharm.
University of Uyo
Nigeria

2014 – 2015.....Intern Pharmacist
University of Uyo
Teaching Hospital,
Uyo, Nigeria

2015 – 2016.....Hospital Pharmacist
Chronic Disease Clinic,
Abia, Nigeria

2016 – 2017.....Pharmacy Manager
Baykins Pharmacy,
Lagos, Nigeria

2017 – 2020.....Pharmacy Manager
Medplus Pharmacy,
Lagos, Nigeria

2020 – 2021.....Medical Liaison
Swiss Pharmaceuticals,
Lagos, Nigeria

2022 - 2024 .....Graduate Research Assistant
College of Pharmacy and
Health
Sciences, Texas
Southern University,
Houston, Texas

Major Field .....Pharmaceutical Science

DEDICATION

This is dedicated to God Almighty, my dear wife, and my beloved parents, whose immeasurable love and support have been consistent and highly appreciated.

ACKNOWLEDGMENTS

I would first like to thank my adviser, Dr. Song Gao, for all his help and guidance during my research study. I am incredibly appreciative of the opportunity to work under your direction. I will use the knowledge you have instilled in me to navigate my professional career successfully. I am immensely grateful to my thesis committee members, Dr. Huan Xie, Dr. Yun Zhang, and Dr. Mario Hollomon, for their indispensable guidance and support in this project. I also wish to thank my lab manager, Dr. Ting, and former and current lab mates, Dr. Sunsong, Dr. Etim, Nyma, Shalom, Eric, and other GPPS, for their help and support when I called upon them. I want to thank my wife Tabitha, my parents, Mr. & Mrs. Sunday Akpan, and friends. Thank you for your unwavering support and love.

CHAPTER 1

INTRODUCTION

Medication-induced diarrhea, especially immunosuppressant drugs, is implicated in the incidence of medication-induced diarrhea, although the pathophysiology is a relatively less studied area. On exposure to the GI tract, the immunosuppressant medication often results in disruption and dysregulation by multiple mechanisms, such as mucosal permeability, motility, and gut microbiota metabolism (Jia et al., 2018). Since several immunosuppressant agents tend to have some form of diarrhea, our focus is on Mycophenolate Mofetil, also known as MMF.

MMF is a prodrug that produces MPA (the active metabolites). MPA preferentially depletes guanosine nucleotides in T and B lymphocytes, inhibiting their proliferation, cell-mediated immune responses, and antibody formation. MPA also suppresses the glycosylation expression of adhesion molecules along with the recruitment of lymphocytes and monocytes into sites of inflammation (Allison, 2005; Parfitt et al., 2008a; van Gelder & Hesselink, 2015). Mycophenolate Mofetil-administered patients reported both acute and persistent diarrhea. The muscarinic function of mycophenolate mofetil is primarily responsible for the early phase of acute onset diarrhea that patients experience, characterized by increased salivation, lacrimation, diarrhea, and diaphoresis. Similarly, mycophenolate mofetil-induced late chronic onset diarrhea is unexpected and can happen at any dose.

Diarrhea is commonly experienced in about 40 patients taking immunosuppressants, and 32.9% of those are MMF-induced. Percent experiencing severe diarrhea (Wieland et al., 2000).

Mycophenolate Mofetil, or 7-ethyl-10-hydroxy-camptothecin, is a prodrug generated from the alkaloid camptothecin. Camptothecin can exist in lactone and carboxylate; their balance depends on PH. The enzyme Serine esterase, also called human carboxylesterase 1 (CES1) and carboxylesterase 2 (CES2), is responsible for hydrolyzing amide and ester linkages in various medicinal goods. Several prevalent genetic variations of the CES1 and CES2 genes have been demonstrated to affect medication metabolism and clinical consequences (Merali et al., 2014).

The active drug, mycophenolic acids, further undergo conjugation in the presence of UGT to the inactive form MPAG (Xu et al., 2012). Enterohepatic recycling occurs when beta-glucuronidase, produced in the gastrointestinal system, deconjugates the conjugated form of MPAG back to the active form of MPA. Studies indicate that oxidative stress and impaired mitochondrial function may mediate MPA-induced intestinal barrier disruption both in vivo and in vitro following prolonged exposure to the GIT (Deng et al., 2022).

Intestinal exposure to MPA may result from several processes, including intestinal CE activity, which converts mycophenolate mofetil to MPA in the intestine, and beta-glucuronidase (GUS) activity, which deconjugates MPAG back to MPA. Since inflammatory mediators promote tissue damage and mucositis, it has been hypothesized that they significantly contribute to the incidence of diarrhea (Stumpf et al., 2022). The loss of normal tissue etiology and villi caused by this intestinal injury causes apoptosis in

the intestinal lumen's crypts, which is clinically manifested as diarrhea and cramps in the abdomen. Although several medications, including atropine, loperamide, and octreotide, have been given to treat diarrhea, problems persist because the body does not always respond to treatment (Bullingham et al., 1996; Nakamura et al., 2001).

MPA is majorly excreted via glucuronidation by converting the phenol group to the inactive 7-hydroxy-β-glucuronide, also known as mycophenolic acid glucuronide (MPAG). A cytochrome P450 catalyzed oxidation product, 7-O-glucoside, and acyl glucuronide have recently been identified as minor metabolites of MPA in humans. The unchanged MPA has minimal renal clearance. Based on MPA and MPAG pharmacokinetic data, renal and gut wall MPA glucuronidation has been hypothesized despite the liver being thought to be the primary source of MPAG production (Bowalgaha & Miners, 2001). Also, Glucuronidation has a significant effect on the physiological characteristics of the flavonoid, including its increased solubility, bioactivity, bioavailability, intracellular and intercellular transit, and excretion (typically increased). The conjugation of flavonoid molecules not only facilitates their absorption, but the location of glucuronidation influences the flavonoids' pro- and antioxidant characteristics (Docampo et al., 2017).

Enterohepatic recycling is known to affect both MMF and flavonoids; monitoring the tissue distribution is imperative. According to reports, mycophenolic acid (MPA) enterohepatic recirculation (EHR) varies greatly, ranging from 5% to 44% and a median of 22%. In the same study, it was observed that MPA EHR was not consistent. Compared to patients with low MPA EHR, those with high MPA EHR showed higher MPA exposure and more Bacteroides species in their feces. Therefore, in certain people,

Bacteroides may be protective against unfavorable outcomes like graft-versus-host disease, but in others, it may raise the likelihood of unfavorable effects from MPA (Saqr et al., 2022). With this in mind, we hypothesize that inhibition of the process of EHR would lead to upregulation or downregulation of certain enzymes like GUS and UGT, which would help reduce or eliminate GI toxicities effectively. Hence, we carried out two specific aims to test our hypothesis. First, Determine the effectiveness of two flavonoids, Wogonin and chrysin, in curtailing MMF-induced diarrhea. Second, we measured the PK parameters to determine the influence of the flavonoids on the MPA produced from the MMF administered.

CHAPTER 2

LITERATURE REVIEW

2.1 Medication-Induced Diarrhea

2.1.1 Overview

A decrease in stool consistency along with an increase in the frequency of bowel movements to between three or more times per day (or the passage of at least 200 g of stool per day by an adult) are defined as diarrhea (Guerrant et al., 2001; Thielman & Guerrant, 2004). Patients who have diarrhea shortly after beginning a new medicine are often suspected of having drug-induced diarrhea (Abraham & Sellin, 2019). Approximately 7% of all medication side effects include diarrhea, making it a relatively common adverse event. Many drugs have been linked to diarrhea; the most common ones are antimicrobials, laxatives, antacids that contain magnesium, products that contain lactose or sorbitol, nonsteroidal anti-inflammatory drugs, prostaglandins, colchicine, antineoplastics, antiarrhythmic medications, cholinergic agents, as well as immunosuppressants (Chassany et al., 2000) and of particular interest are patients who develop diarrhea during a solid organ transplant.

2.1.2 Incidence of Diarrhea in Organ Transplant Patients

According to a 2008 analysis of the 41,442 renal transplant recipients in the US included in the US Renal Data System Database, a three-year cumulative incidence of diarrhea has been 22%, with 18% of such cases being categorized as non-infectious

(Bunnapradist et al., 2008). Mycophenolate mofetil is the most frequent medication that causes diarrhea in patients receiving solid organ transplants (MMF). According to certain studies, up to 51% of liver transplant recipients who take 3 grams of MMF daily experience diarrhea (Ginsburg & Thuluvath, 2005). The diagnosis, prevention, and treatment of diarrhea during the pre-and post-transplant period are reviewed in guidelines from the American Society of Transplantation

Infectious Diseases Community of Practice. An organ transplant recipient experiencing diarrhea runs the risk of serious complications such as dehydration, increased medication toxicity, and rejection. (Angarone & Snydman, 2019) There are many different causes of diarrhea in transplant recipients, but the most frequent ones include infections, especially clostridium difficile and norovirus, as well as medication-induced diarrhea. Although much less prevalent, additional bacterial, viral, and parasite sources can cause diarrhea. Moreover, diarrhea in the transplant population can also be caused by non-infectious factors such as inflammatory bowel disease, post-transplant lymphoproliferative disease, drug toxicity, as well as cancer.(Angarone & Ison, 2015)

2.1.3 Drugs Used in Solid Organ Transplant

There are two categories of drugs used in organ transplants: Induction agents and maintenance agents. Induction agents are potent antirejection drugs administered during transplantation, while maintenance agents are antirejection drugs taken over an extended period(Sutherland et al., 2016).

Maintenance agents are often classified into four categories:

- Calcineurin Inhibitors such as Tacrolimus and Cyclosporine
- Antiproliferative agents such as Mycophenolate mofetil

- Mammalian target of Rapamycin(mTOR) inhibitor like Sirolimus
- Steroids: Prednisolone.

For maintenance purposes, Physicians typically recommend a combination regimen because the various medication classes have distinct adverse effects and modes of action. High levels of immunosuppression are achieved with the combination strategy without allowing any one sort of adverse effect to worsen than it otherwise might. Depending on the recipient's and the graft's immunologic compatibility, different levels of immunosuppression are required. Doctors typically cut immunosuppressive drug dosages empirically as transplant recovery times grow. They experiment with little adjustments to see whether adverse effects may be minimized without endangering graft rejection. Immunosuppressive medications have several obvious advantages for transplant recipients: increased longevity and better quality of life(Cossart et al., 2019)

2.1.4 Calcineurin Inhibitors

Calcineurin Inhibitors (CNIs) are the cornerstone of immunosuppression for organ transplant. The two medications that make up this class, tacrolimus (TAC) and cyclosporine A (CsA), have been administered to kidney transplant recipients for about 30 years. The Food and Drug Administration (FDA) of the United States authorized using CsA in 1983 for immunosuppression after organ donation; in 1995, a microemulsion formulation of CsA, linked to improved bioavailability and more consistent absorption, was allowed. Typically, CsA formulations are taken twice a day. FDA approved TAC in 1994 for patients of liver transplants and in 1997 for recipients of kidney transplants. Although tacrolimus is typically taken twice daily, an extended-release version is now offered once daily(Matas et al., 2015). Off-label, topical tacrolimus is also used to treat

pediatric genital and facial psoriasis(Menter et al., 2020). Nephrotoxicity, hypertension, gingival hyperplasia, hypertrichosis, hepatotoxicity, hyperkalemia, and neurotoxicity are only a few of the adverse effects that CNIs have been linked to (Ahmed et al., 2021).

2.1.5 Mammalian target of Rapamycin(mTOR) inhibitors

This class is a well-established component of the immunosuppressive toolkit used after organ transplants. This class has been thoroughly studied in the kidney (Peddi et al., 2013), heart (Manito et al., 2010), and liver transplantation (Asrani et al., 2010). The most common members of this category are Sirolimus and Everolimus (Klawitter et al., 2015). The common side effects of this family include Mucositis and rash (Soefje et al., 2011).

2.1.6 Steroid usage in Organ transplants

Since glucocorticoids (GCs) have strongly affected innate immunity and tissue protection, they have been the cornerstone of immunosuppressive therapy used in solid organ transplantation (SOT) for many years. However, because of the numerous associated side effects, some SOT clinics are hesitant to provide GCs for an extended period. Steroids enhance the risk of bacterial, fungal, and viral infections in transplant patients (Smith, 2003). It also causes reduced absorption of calcium in the interstitial area, inhibits growth hormone secretion, and generally inhibits osteoblast differentiation, which leads to inhibition of bone formation (Ponticelli & Glassock, 2019).

2.1.7 Antiproliferative agent

Mycophenolic acid and azathioprine, which are less frequently used until under specific situations, are examples of antiproliferative drugs. Their significance in disrupting cellular replication led to their first identification for application in

autoimmune and cancer research. These days, they serve as the mainstay of solid organ transplant antirejection maintenance therapy(Donovan et al., 2021). Antiproliferative drugs have potential benefits in preventing or lowering Chronic Allograft Nephropathy (CAN) come from the antiproliferative actions on cells like fibroblasts and vascular endothelium, but these same effects are also likely to be the cause of impaired renal function, poor wound healing, and bone marrow suppression(Fletcher et al., 2009)

The major challenge with this group is also drug-induced diarrhea, particularly with the first-line treatment in most autoimmune diseases, and prevention of organ rejection is medication-induced diarrhea.

2.1.8 Mycophenolate mofetil (MMF or Cellcept)

Mycophenolate mofetil, MMF, and CellCept are prodrugs of mycophenolic acid and are categorized as IMPDH (inosine monophosphate dehydrogenase) reversible inhibitors.

2.1.9 Mycophenolic Acid (MPA)

Mycophenolate mofetil, which is a prodrug of MPA. Following Oral administration of MMF, it undergoes a rapid presystemic bioactivation to MPA by the enzyme carboxylesterases (CES), particularly CES-1 and CES-2(Satoh et al., 2002). In systemic circulation, MPA has a mean half-life of roughly 17 hours. The majority of MPA's metabolism occurs in the liver, where it is glucuronidated to produce the primary metabolite, 7-O-glucuronide metabolite (MPAG), which is pharmacologically inactive, and the active metabolite, MPA and MPA-acyl-glucuronide (AcMPAG), which is accountable for the GI harmful effects. Approximately thirty-five percent of the MPA area under the curve (AUC) is attributed to enterohepatic circulation. Following injection,

a subsequent plasma peak appears 6–12 hours later. This enterohepatic route for MPA is inhibited by cyclosporin, which lowers total MPA plasma levels, hence why they, though harmful, are used together. Eventually, the kidneys eliminate MPA via MPAG(Jeong & Kaplan, 2007).

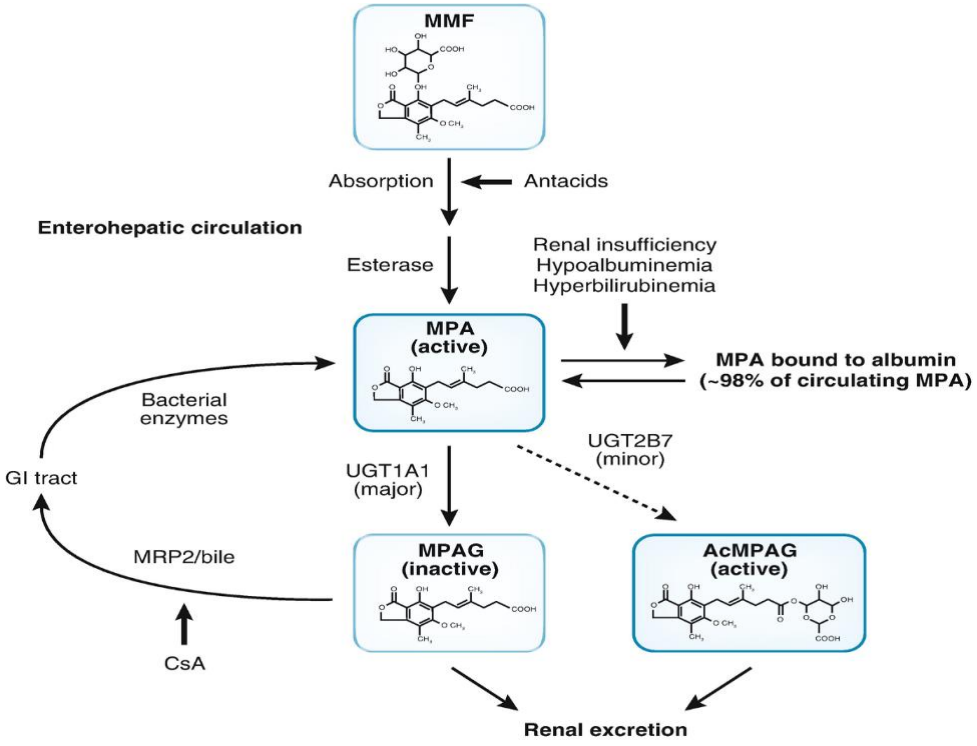

Figure 1. Pharmacokinetics of MMF. MMF Mycophenolate mofetil, MPA Mycophenolic acid, MPAG inactive 7-O-glucuronide metabolite, AcMPAG active metabolite MPA-acyl-glucuronide. From Therapeutic Monitoring of Mycophenolate Mofetil by Hyunyoung Jeong and Bruce Kaplan. CJASN January 2007, 2 (1) 184–191. R (Jeong & Kaplan, 2007)

2.2 Chemical nature and GI toxicity mechanism of Mycophenolate Mofetil

Mycophenolate mofetil has the following chemical properties:

- Molecular Weight: 433.5 g/mol

- Dissociation Constants: pKa 5.6 for the morpholino group and 8.5 for the phenolic group
- LogP: log Kow = 2.38 at pH 7.4
- Solubility: Is freely soluble in acetone, soluble in methanol, and sparingly soluble in ethanol, and in water, 43 mg/L at pH 7.4
- Chemical structure along with metabolites:

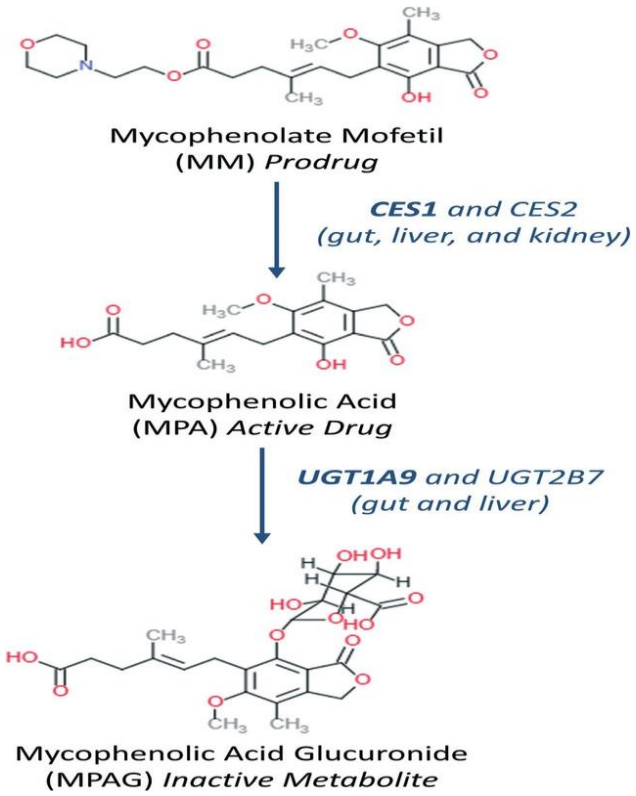

Figure 2. Chemical structure of MMF, MPA and MPAG

Source: Lab Chip, 2022, 22, 2853 ((Milani et al., 2022)

MMF is approved for treating various solid immune system disorders and stomach, lung, and colorectal malignancies. As stated before, as a prodrug, mycophenolate mofetil, a camptothecin derivative, is hydrolyzed to MPA. This

functional moiety is responsible for inhibiting topoisomerase-1 activity during DNA replication processes in the presence of CES. Mycophenolate Mofetil causes both acute diarrhea, which happens right after dosage administration, and chronic diarrhea, which happens later in life. Increased intestinal exposure to the active ingredient in MPA in the GI tract causes diarrhea. However, there are still difficulties with using mycophenolate mofetil. These GI side effects may be transient or persistent, as previously mentioned. Cholinergic activities, which affect 70–80% of individuals, cause the immediate acute adverse effects. When 125 mg/m2 of mycophenolate mofetil was administered weekly for a median of ten days, diarrhea episodes started to occur within two to three hours(Cardet & Boyce, 2013). The episode of persistent diarrhea happens concurrently. This syndrome is a complex situation involving intestinal secretion and inflammatory mucosa rather than being mediated by a cholinergic response. The dose-limiting adverse effect of this late-stage diarrhea can be significantly increased in elderly or immunocompromised people who also have additional toxicities.

MMF may impact upper and lower GI tracts (Parfitt et al., 2008b). Previous research has examined the colonoscopic and histological characteristics of patients with post-transplantation diarrhea who are on MMF. Twenty colonic biopsies from renal transplant patients with diarrhea associated with MMF were examined by Papadimitriou et al. (Papadimitriou et al., 2003). Biopsies from 24 diarrhea patients receiving MMF treatment were examined by Dalle et al. (Dalle et al., 2005). Nineteen patients exhibited characteristics like Crohn's disease, including angular crypts and crypts dilating to different diameters and neutrophil and macrophage infiltration. They could not find any concrete evidence linking the histological alterations and diarrhea. Nonetheless,

pathologists should not ignore colitis in MMF-treated transplant patients as it is a unique condition because a false positive could alter the course of treatment.

Three primary diarrhea pathophysiological mechanisms are watery, fatty, and aggressive.

Secretory and osmotic diarrhea are other classifications for watery diarrhea. while excretory diarrhea is caused by a disruption in epithelial electrolyte transport, which causes an increase in electrolyte discharge. For instance, Mycophenolate Mofetil, when co-administered with other toxic compounds, such as Cyclosporine, may harm the intestinal mucosa, leading to aberration and the loss of intestinal epithelium (Yu et al., 1998).

2.2.1 Mycophenolate Mofetil disposition: Bacterial Beta GUS activity and Enterohepatic Recirculation

MMF has a complicated metabolism and distribution. Enterohepatic recirculation (EHR), mediated by intestinal b-glucuronidases primarily generated by gram-negative anaerobes, occurs in MPA (Staatz & Tett, 2007). EHR is significant because it helps to keep MPA blood concentrations stable. Variability in the EHR across and among patients may result in erratic immunosuppression, which could have a negative impact on MMF toxicity, GVHD protection, and engraftment. Microbiome-derived metabolism has been found in 28 pharmacologic classes, including MMF, using ex vivo culturing systems of microbial communities. This metabolism leads to the parent drug's depletion or novel metabolites' emergence (Javdan et al., 2020). MMF was one of the most intensively metabolized medications in an in vitro microbiome model, with about 70 microorganisms exhibiting metabolic activity (Zimmermann et al., 2019)

Using experimental data, Zimmerman-Kogadeeva et al. (Zimmermann-Kogadeeva et al., 2020) recreated host-microbiome deglucuronidation and glucanosyltransferase enzyme activity and examined the circumstances in which EHR influences drug exposure. Using these models, they could forecast medication concentrations, demonstrating the possible involvement of the microbiome in the pharmacokinetics of MMF. MMF is known to cause diarrhea, but a higher fecal b-glucuronidase activity in kidney transplant recipients is linked to a longer course of diarrhea compared with lower b-glucuronidase activity, and this shows that any activity that reduces the population of the bacteria producing diarrhea or inhibits the bacteria will lead to reduced or complete elimination of diarrhea, hence in this study we will attempt to use flavonoids.

2.2.2 Management of Mycophenolate mofetil-induced diarrhea:

Discontinuation of the drug is the most common action, followed by weight reduction. According to a study where 560 episodes of diarrhea induced by MMF were understudied, 56% of patients had MMF stopped or had their dosage lowered; 12% had moved to enteric-coated mycophenolate mofetil sodium, and 14% had continued with MMF.85% of individuals who received MMF treatment (discontinuation or dose decrease) and eighty-one percent of cases who received enteric-coated mycophenolate mofetil sodium instead of MMF responded better(Dhakal et al., 2017). However, these withdrawals and dose reductions often cause overall organ rejection or graft-versus-host disease (GVHD). We hope to use flavonoids in the experiment: Wogonin and Chrysin.

2.2.3 Flavonoids

Plants contain naturally occurring substances called flavonoids, which have varying phenolic structures. There has been much research done to find flavonoids with new therapeutic applications. Chemically, flavonoids are composed of a fifteen-carbon skeleton that is connected via a heterocyclic pyrane ring (C) to two benzene rings (A and B,). Flavonoids can be separated into numerous classes.

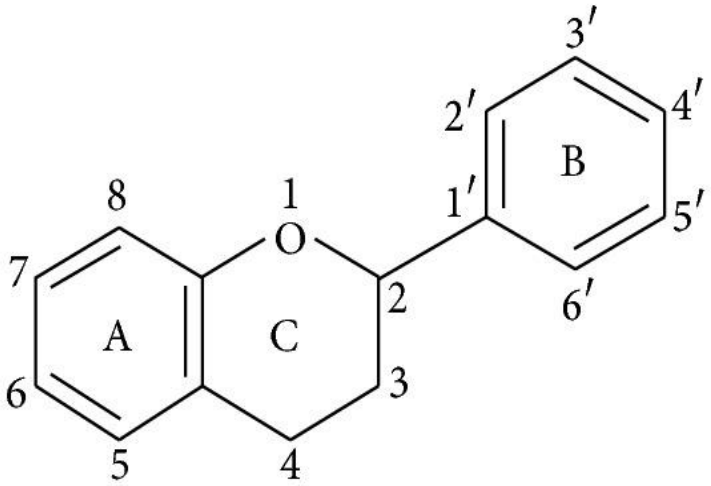

Figure 3. Backbone structure of Flavonoids (Source: Scientific World Journal. 2013; 2013: 162750) (Kumar & Pandey, 2013)

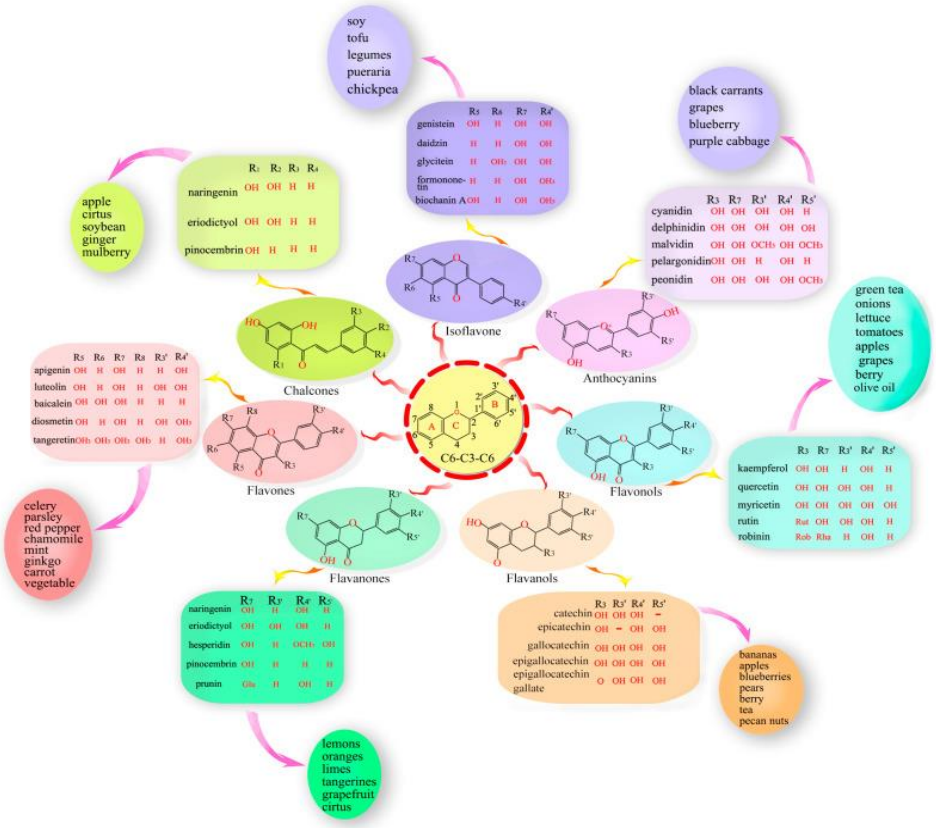

Figure 4. Classification of flavonoids based on chemical nature with examples. Source: Molecules. 2023 Jul; 28(13): 4982 (Chen et al., 2023)

Flavonoids are known to have several Pharmacologic properties, which include:

- Antioxidant activities: The position and quantity of hydroxyl groups in flavonoids' structures and their stable roles in various matrices determine their antioxidant activity. The three processes listed below comprise most of the mechanisms of antioxidant activity: (1) Scavenging ROS directly. Flavonoid antioxidant activity in vitro is contingent upon the configuration of hydroxyl groups. Because of the ortho-dihydroxy structure of the B-ring, flavonoid phenoxy radicals can engage in electron delocalization, which causes an electron

in the B-ring to dislocate and exhibit antioxidant activity. The antioxidant activity of these structures is increased. (2) The antioxidant enzymes' activation. Certain flavonoids can inhibit the functions of enzymes that produce free radicals, like xanthine and nitric-oxide synthase(VICENTE & BOSCAIU, 2018)

- Antimicrobial activity: Most research on antiviral substances inhibits several enzymes connected to the viral life cycle. It has been noted that there is a structure-function relationship between flavonoids and their ability to inhibit enzymes. Flavan-3-o1 was shown by Gerdin and Srensso (Gerdin & Svensjö, 1983)to be more successful in selectively inhibiting HIV-1, HIV-2, and related immunodeficiency virus infections than flavones and flavanones. Scutellaria baicalensis (Lamieaceae) is the source of the flavonoid baicalin, which prevents HIV-1 infection and replication. It has also been demonstrated that hinoki flavone and robusta flavone, among other flavonoids, block HIV-1 reverse transcriptase(Cushnie & Lamb, 2005).
- Pain Management: Flavonoids have antioxidant, analgesic, and anti-inflammatory qualities. These outcomes are associated with suppressing pro-inflammatory cytokines (Borghi et al., 2018) that depend on NF-κB, VEGF, ICAM-1, and STAT3 (Verri et al., 2012).
- Others include anticancer(Zhao et al., 2019), antibacterial, antiviral, antiangiogenic, antioxidant, antimalarial, neuroprotective, antitumor, and anti-proliferative drugs, flavonoids have been widely utilized. Rich in flavonoids, apple peel extracts effectively lower blood pressure by inhibiting acetylcholinesterase (ACE) in vitro(Khan et al., 2018). Moreover, it guards

against cardio-metabolic diseases and shows improved cognitive function preservation as one ages (Aguiar et al., 2019).

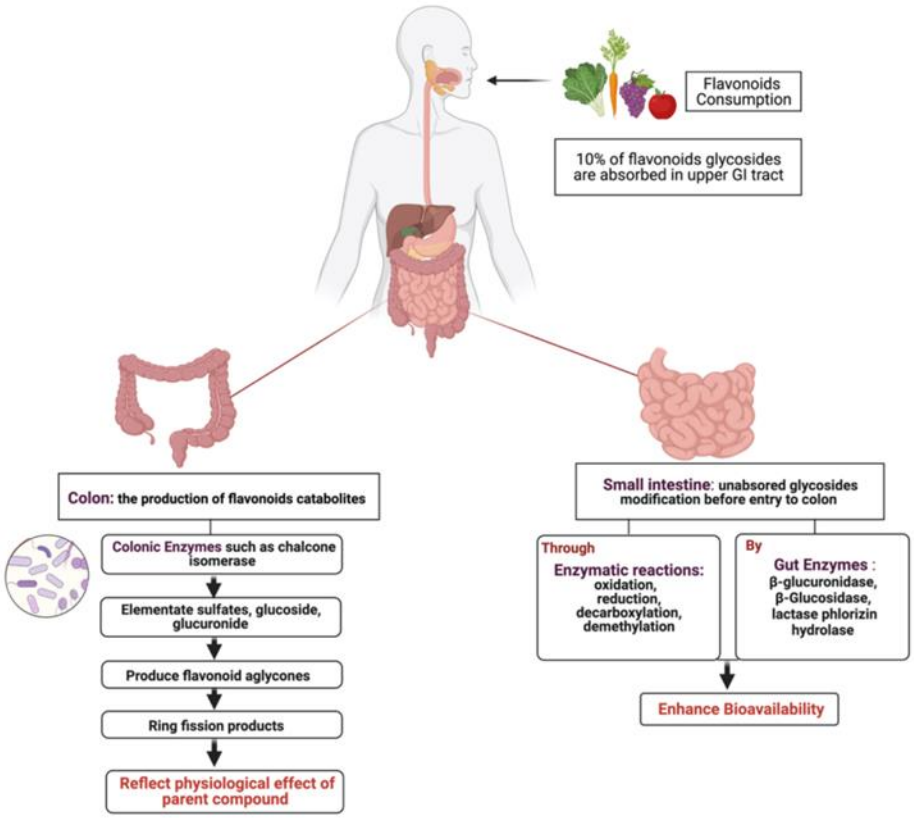

Figure 5. Metabolism of flavonoids, which explains enhanced local bioavailability of Wogonin in the intestine and colon.

Source: Cancers 2021, 13(16), 3934 ((AL-Ishaq et al., 2021a)

2.2.4 Wogonin

With the molecular structure 5,7-dihydroxy-8-methoxyflavone, wogonin is found exclusively in Lamiaceae and Scutellaria species. One of the main ingredients in the root extract of *Scutellaria baicalensis*, used for hundreds of years in Chinese traditional medicine, is wogonin. Research in vivo and in vitro have shown the therapeutic efficacy of wogonin. Its sedative, antiviral, anticancer, and anti-inflammatory properties stand out in particular(Aguiar et al., 2019)

Most xenobiotics undergo both enterohepatic and enteric recycling mechanisms in the intestinal tract. However, wogonin is known to undergo a third recycling mechanism for glucuronides: local recycling, which further enhances its local bioavailability. The local recycling of wogonin and wogonoside is the first evidence that this unique mechanism functions in the upper small intestine without a large input from bacteria β-glucuronidase (Xia et al., 2012).

2.2.5 Chrysin

Chrysin is a dietary phytochemical widely found in various plants. Its bioavailability and absorption mean that research on its medicinal advantages is still in the early stages.

Chrysin reduces oxidative stress, neurotoxicity, and neuroinflammation. The enzymes that break down chrysin have a strong affinity for the molecule. Its bioavailability needs to be increased to utilize the full potential of chrysin as a medicinal chemical.

CHAPTER 3

DESIGN OF STUDY

3.1 General Hypothesis

We hypothesize that Mycophenolate mofetil-induced diarrhea can be mitigated by inhibiting the enterohepatic recycling process without significant changes in the Pharmacokinetics of the active drug mycophenolic acid using wogonin and chrysin. The inhibition is anticipated to enhance gastrointestinal glucuronidation of mycophenolic acid (MAPG), facilitating the elimination of more hydrophilic and less toxic mycophenolic acid glucuronide (MPAG). By modulating these pathways, we aim to alleviate gastrointestinal side effects associated with MMF treatments.

3.2 Specific Aims

3.2.1 Specific Aim 1

To investigate the effectiveness of the flavonoids Wogonin and Chrysin in eliminating MMF-induced diarrhea.

Rationale

The aim is to study the toxicity of mycophenolate mofetil in rats, with particular emphasis on the gastrointestinal tract (the colon and small intestine) and liver tissue concentrations.

Experimental Design.

We hope to evaluate the diarrhea score index by measuring the body weight, survival rate, and fecal diarrhea score of a female Wistar rat.

3.2.2 Specific Aim 2

To Characterize the Pharmacokinetic profile of wogonin and chrysin when administered along with Mycophenolate Mofetil.

Rationale

Mycophenolate mofetil's disposition is less understood, and several investigations have been made to alleviate diarrhea while maintaining reasonable pharmacokinetics. We believe that if the Pharmacokinetics are altered, the systemic effects of MMF will be reduced.

Experimental Design

We collect plasma blood samples from the animal's tail for PK evaluation and organ tissues from the GIT and Liver. The rats were grouped as control (administered only MMF), treatment with Wogonin + MMF, and Treatment with Chrysin + MMF only. We quantify both the plasma and tissues using LCMS.

3.3 Materials

3.3.1 Chemicals and Drug Compounds

- MMF, MPA, AcylMPAG MPAG, Chrysin, and wogonin were purchased from Sigma-Aldrich
- The ELISA kit was bought from Abcam (Waltham, MA).
- Deionized water, as well as the dispenser were bought from Thermo Fisher Scientific.
- HPLC/LCMS- grade acetonitrile and water were purchased from Sigma Aldrich
- Formic acid was purchased from Sigma Aldrich.

3.3.2 Supplies

- Microcentrifuge tubes (1.5 mL amber and clear from VWR) were used to prepare samples, including working standard solutions.
- Pipettes were bought from VWR (Radnor, PA).
- Two-mL autosampler injection vials were purchased from Agilent (Santa et al.) and used to fill samples for injection in the LCMS machine.
- Pipette tips (ranging from 0.1 µL up to 5000 µL)
- Latex examination gloves.
- Beakers were purchased from VWR (Radnor, PA)
- Storage bottles for LCMS were purchased from VWR (Radnor, PA).

3.3.3 Apparatus, Equipment, and Software

- The Analyst software was used alongside the LCMS system.
- GraphPad Version 7.3 for windows was used for statistical analysis.
- Magnetic stirrer (PC-351)
- A multimode detector DTX 880 was purchased from Beckman Coulter to read the absorbance of samples.
- A minus 80 °C refrigerator from Thermo Fisher Scientific was used to store the biological samples after collection.

3.3.4 Animals

The Texas Southern University IACUC (Institutional Animal Care and Use Committee) approved their animal protocol review. The Rats were allowed to get used to the vivarium for 10 days before animal experiments. The rats were selectively randomized according to their body weights.

3.4 Methods

3.4.1 Diarrhea model and efficacy study

Wistar Han Rats were used after being fed for about ten days upon arrival at the animal facility. The animal species used for the experiments were the female Wistar Han rats, which were divided into three main groups: the control (MMF only), treatment group A(Wogonin plus MMF), and Treatment group B (Chrysin plus MMF).

The treatment groups were orally dosed with wogonin and chrysin consecutively for two days before commencing the seven-day MMF dosing. Similarly, the control group was only administered MMF for seven days.

The body weight and feces of rats were monitored daily to observe for the diarrhea index score. Daily, the animals was observed for the presence of diarrhea which can be graded 1-4 (Grade 1 – soft stool, grade 2 – semisolide stool, Grade 3- watery stool, and Grade 4 – bloody stool). The animals were checked for diarrhea score levels from day 3 to day 9. On day 9 of the experiment, blood samples were taken from the Rat's tail in heparinized tubes for processing into plasma. On day 10, the surviving rats were euthanized using isoflurane, while tissues, including the liver, small intestine, and colon, were collected. The weight of the tissue was measured. Also, to evaluate the drug's concentration, the tissue concentration was quantified using LC-MS/MS while detecting the concentration

of MMF, MPA, MPAG, and AcylMPAG, respectively.

3.4.2 Protein assay

The protein concentrations were ascertained using a BCA kit and the Bradford technique, and the manufacturer's instructions were followed throughout the process.

3.4.3 Tissue drug quantification using LC-MS/MS

Shortly, 200 mg of tissue samples were weighed and placed into 2 mL centrifuge tubes. Each tube was filled with 0.4 mL of water using a tiny steel ball. The samples were then homogenized for a minimum of 30 to 60 seconds. Then, 1 mL of acetonitrile, the extraction solvent, was added. It was then sonicated for 5 minutes and centrifuged for at least 15 minutes at 14,000 rpm and 4°C. Next, a fresh 2 mL centrifuge tube containing roughly 80% of the supernatant was pipetted and allowed to air dry. After that, the residue was again air-dried after being reconstituted with 0.1–0.2 mL of 90% acetonitrile (ACN) to precipitate the protein. Following another centrifugation, 200 uL of 50% ACN is used to reconstitute the residue. The supernatant is then injected for LC-MS/MS analysis.

3.4.4 Plasma drug quantification using LC-MS/MS

To quantify, 180 uL of extraction solvent (ACN) and 20 uL of plasma were combined, vortexed, and sonicated for five minutes. After 15 minutes of centrifugation at 14,000 rpm and 4°C, 80% of the supernatant was removed for air drying, and 200 uL of 50% ACN was added and centrifuged once more after air drying. The supernatant was then injected for LC-MS/MS analysis. After that, 180 uL of extraction solvent (ACN) was mixed with 20 uL of plasma, vortexed, and sonicated for approximately five minutes. After 15 minutes of centrifugation at 14,000 rpm and 4°C, 80% of the supernatant was

removed for air drying. 200 uL of 50% ACN was centrifuged once more after air drying, and the supernatant was then injected for LC-MS/MS analysis.

Statistical Analysis

The statistical differences were assessed using a two-tailed t-test. GraphPad Prism (version 7.3 for Windows) was used to make statistical comparisons, and differences deemed significant were those with p-values less than 0.05.

CHAPTER 4

RESULTS AND DISCUSSION

Specific Aim 1. Evaluate the efficacy of Wogonin and Chrysin in alleviating Mycophenolate mofetil-induced diarrhea.

4.1 Evaluating the Efficacy of Wogonin and Chrysin

4.1.1 Oral flavonoids Wogonin and Chrysin alleviate MMF-induced diarrhea in rats.

The diarrhea model was earlier established in a previous study in the laboratory, which shows the appropriate dose and food required for the diarrhea model; hence we delved into the experiment by dividing the animals into three batches: A control batch (Mycophenolate mofetil only), treatment batch A (Mycophenolate mofetil co-administered with wogonin), and treatment batch B (Mycophenolate mofetil co-administered with chrysin). An oral dose of wogonin and chrysin (100 mg/kg) consecutively was administered to the Wistar rats seven weeks old over two days before beginning Mycophenolate Mofetil (70 mg/kg) orally on day three. This was repeated for 50mg/kg on a different batch of animals that were 11 weeks old at the time. Several parameters were used to measure the attainment of this aim, including the body weight index, diarrhea score index, and survival plot.

4.1.2 Body weight index

The Control group showed a significant loss in weight of the animal when compared to wogonin and chrysin. Wogonin showed a much slower weight reduction process, while chrysin showed a weight loss range slightly lower than the control group. The 50mg/kg/day combined flavonoids group demonstrated a much steeper weight loss, while the 100mg/kg/day group (MMF combined with either Wogonin or Chrysin) had a less steep weight loss. They all had a weight loss of less than 10% compared to the control, which had a weight loss of up to 20%. The body weight was taken at about the same time daily before administering the oral gavage. The animals in the first batch whose treatment group was administered 50mg/kg of the flavonoids had initial weights ranging from 170g to 210g; however, at the end of the experiment, all the weights had drop dropped to between 180-140g for the control group, while the Chrysin group had a much tighter range of weight until day 7. However, the wogonin group all maintained a much more consistent weight change that was insignificant.

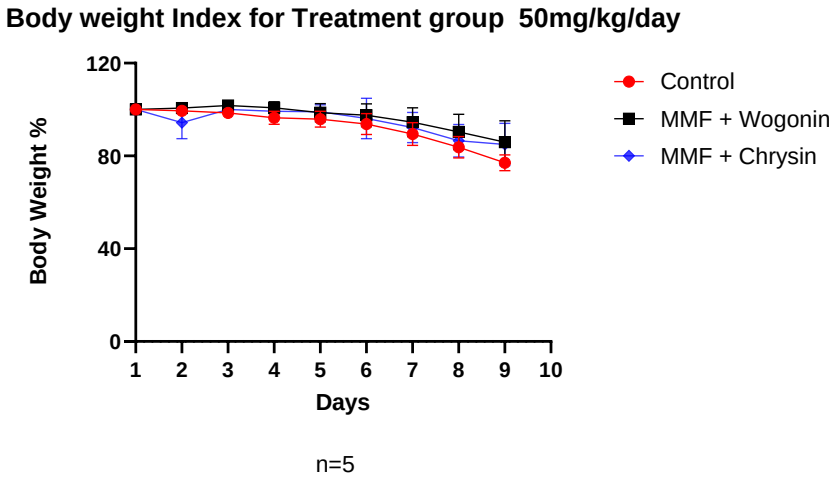

Figure 6. Body Weight index showing percentage weight loss in treatment group 50mg/kg/day

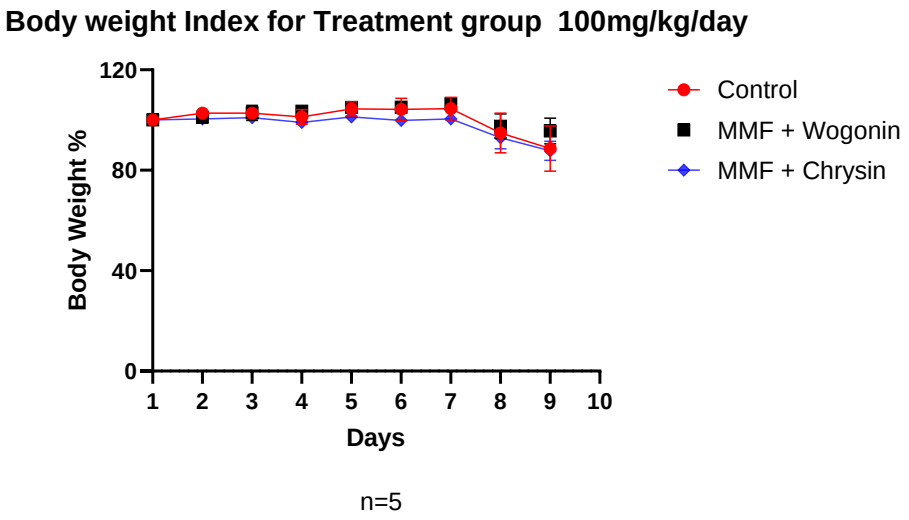

Figure 7. Body Weight index showing percentage weight loss in treatment group 100mg/kg/day

4.1.3 Diarrhea score index

50mg/kg/day treatment group:

The diarrhea score index measures the extent of the diarrhea formed using a scale from Grade 1 to Grade 4. Grade 1 is often difficult to detect, whereas grades 2,3 and 4 are much more apparent. Between grades 1 and 2, the stool was found to be soft; grade 3 had watery stools, and Grade 4 had bloody stools. Watery diarrhea is proposed to be caused by the deconjugation of bile acids resulting from gluten or illness, which also raises cAMP(FAN & SELLIN, 2009)

On day 7 of dosing, the 50mg/kg/day batch started developing diarrhea. The diarrhea score indicates that the control group had grades 1, 3, and 4 between days 7- 10. On the other hand, the combined group, MMF + Wogonin, had an improved diarrhea score, which was indicated by the absence of grade 3 diarrhea on day seven and the overall absence of Grade 4 diarrhea between day seven and day 10. Also, about 60% of

grades 1 and 40% of Grade 2 diarrhea only took place on day seven and only started having Grade diarrhea on day nine compared to the control, which had already begun on day 7. Conversely, it was observed that the Treatment group that had MMF + Chrysin within the 50mg/kg/day treatment batch had about 60% Grade 1, 20% Grade 2, and 20% Grade 3 diarrhea on seven as compared to the control which had 80% Grade 3 diarrhea on day 7 and 20% Grade 1 diarrhea.

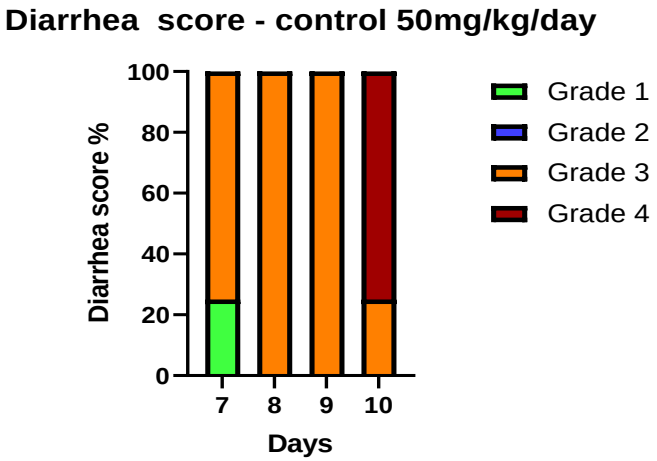

Figure 8. The Diarrhea Score Index of the Control batch dosed MMF 70mg/kg/day only

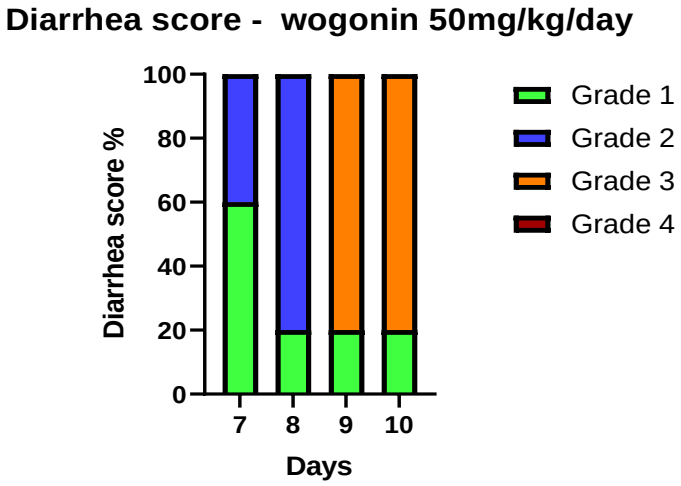

Figure 9. The Diarrhea Score Index of the Treatment batch dosed MMF 70mg/kg/day with 50mg/kg/day of Wogonin.

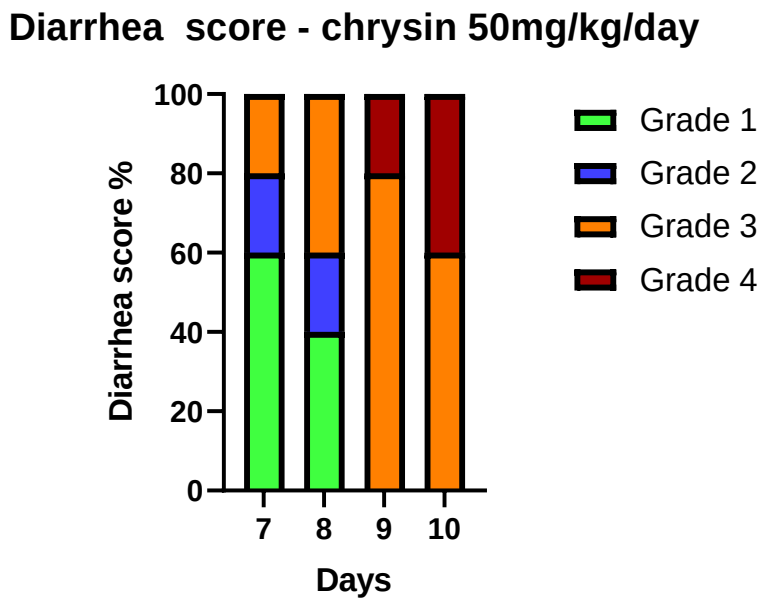

Figure 10. The Diarrhea Score Index of the Treatment batch dosed MMF 70mg/kg/day with 50mg/kg/day of Chrysin

4.1.4 100mg/kg/day batch:

The 100mg/kg/day treatment batch exhibited a corresponding pattern that clearly showed the flavonoids' efficacy in influencing the inhibition of MMF-induced diarrhea. Interestingly, severe diarrhea did not commence in the Control (MMF only) group until day 8, where it had about 60% grade 3, 20% grade 2, and 20% grade 1 diarrhea, respectively. The diarrhea became intense on days 9 and 10, with the animals having 60% grade 3 and 40% grade 4 diarrheas each day. When comparing this to the MMF + Wogonin treatment group, diarrhea started on day 8, with 80% of diarrhea in grade 1 and 20% in grade 2. Also, this group had 60% grades 2 and 40% grades 3 on the 10th day of dosing, clearly showing the combination's efficacy. Nevertheless, the chrysin group never had grade 4 diarrhea, but on days 8-10, it had grade 3 diarrhea, and when compared to the control, it still showed efficacy. This has also been reported in other species besides

rats(Meng et al., 2017a).

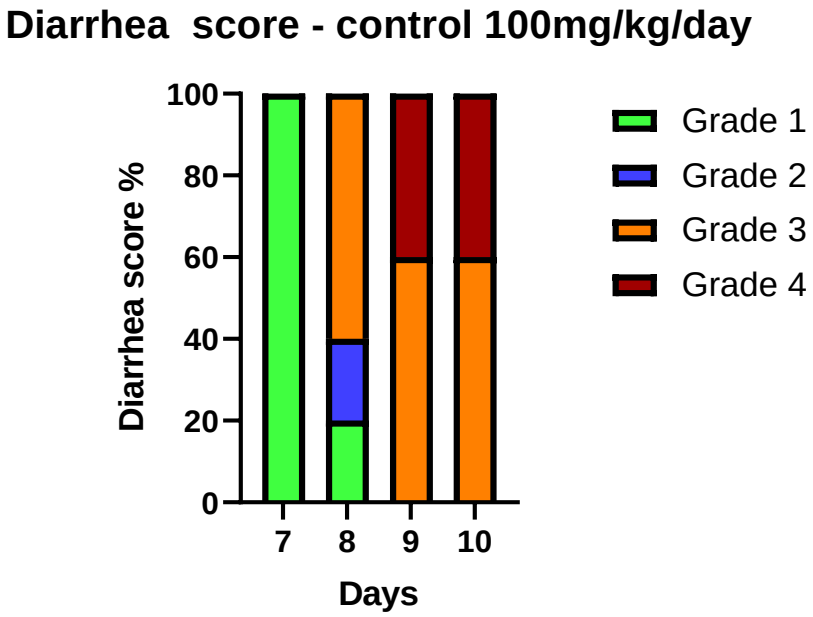

Figure 11. The Diarrhea Score Index of the Control group dosed MMF 70mg/kg/day only

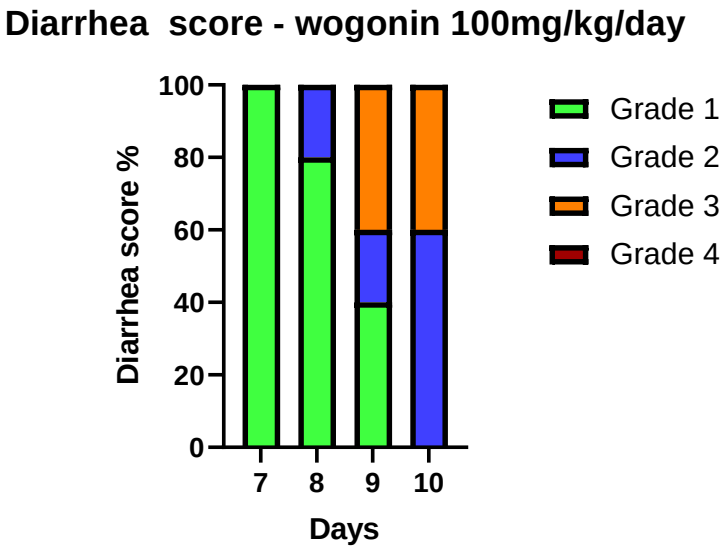

Figure 12. The Diarrhea Score Index of the Treatment batch dosed MMF 70mg/kg/day with 100mg/kg/day of Wogonin.

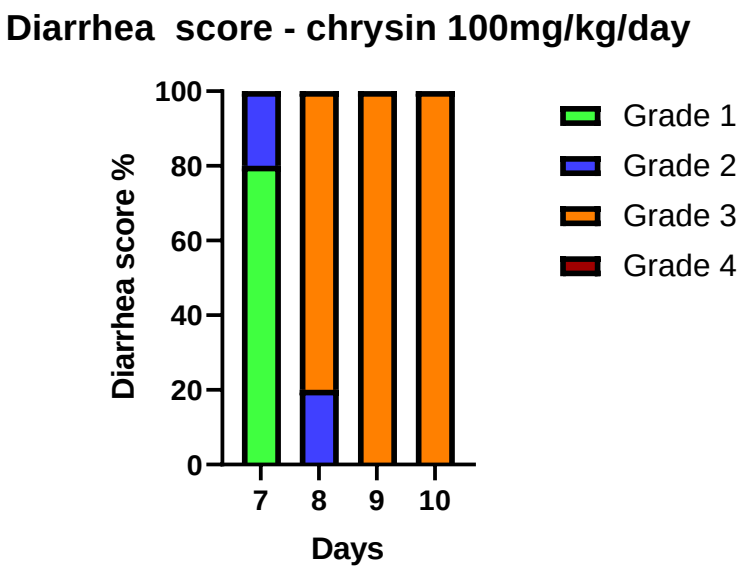

Figure 13. The Diarrhea Score Index of the Treatment batch dosed MMF 70mg/kg/day with 100mg/kg/day of Chrysin

4.1.5 Survival Plots following administration of MMF:

The survival Plot indicated that the Female Wistar rats dosed for the treatment group at 50mg/kg/day did not all survive throughout the experiment, and on the contrary, all the 100mg/kg/day dosed treatment groups survived. As shown by the survival plot, about 2 of the control group died between days 8-10, while none from the wogonin-treated group died. However, the treatment group that had chrysin had a low survival rate and was like the control group. This shows that though Chrysin might have some diarrhea-preventive activity, it does not have a reasonable survival rate. The lack of inhibition of MPA conversion to MPAG in the presence of beta-glucuronidase in the GIT by chrysin may lead to non-survival since, as evidenced in groups with grade 4 diarrhea plots and in line with the clinical data conducted with 150 patients in a randomized, double-blind study to check for safety and efficacy of mycophenolate mofetil used orally to avoid severe rejection following kidney transplantation.(van Gelder et al., 1999)

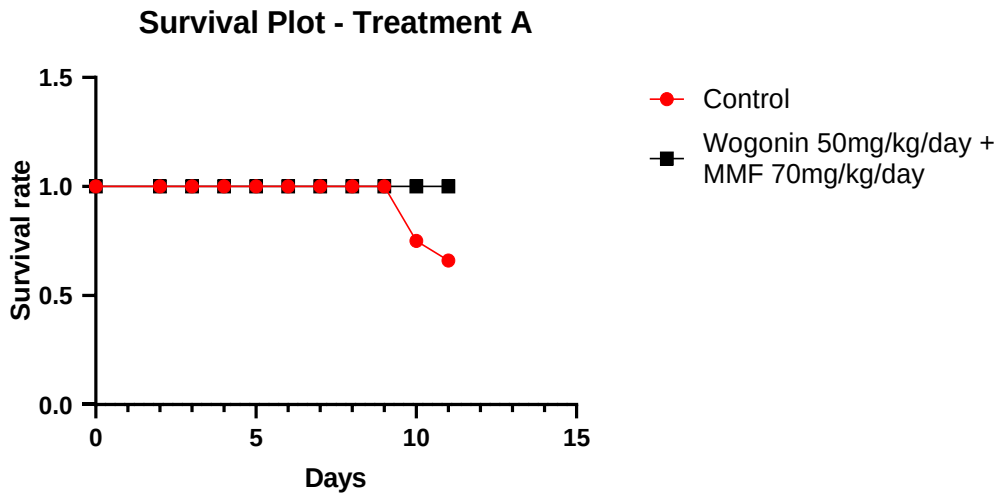

Figure 14. The Survival plot of the Treatment batch dosed MMF 70mg/kg/day with 50mg/kg/day of Wogonin

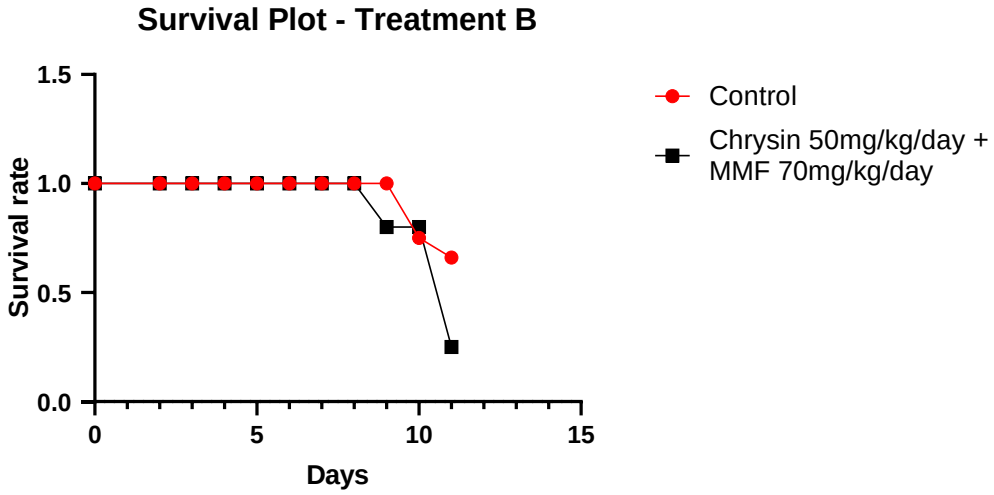

Figure 15. The survival plot of the treatment batch MMF dosed at 70mg/kg/day and 50mg/kg/day of Chrysin

Specific Aim 2. Determine the influence of Wogonin and Chrysin on the Pharmacokinetics profile of Mycophenolate mofetil.

Some drugs expelled from the biliary system are reabsorbed in the intestine rather than removed, a process known as Enterohepatic recycling. A prominent example of a medicine that gets recirculated is MMF. Various gut bacteria produce beta-glucuronidase, and variations in the quantity or makeup of these microorganisms may impact systemic exposure and MMF recirculation. In the short term, mycophenolic acid is essential for effective immunosuppression following kidney transplantation(Ekberg et al., 2007) and even in the long term. (Guerra et al., 2011). To determine the Pharmacokinetic profile in this experiment, we used the WinNonlin software, and a non-compartmental analysis model was used for all the groups,

We used this study to attempt to explain the differences in pharmacokinetic profiles of MMF alone and in combination with Chrysin or Wogonin. Tissues were collected on day ten to determine the effect of wogonin and chrysin on Mycophenolate Mofetil-induced exposure. The concentration of the Mycophenolate Mofetil metabolites MPA and MPAG was determined. From the large batch of samples, the drug concentration of MPA was most significant in the colon for chrysin treatment groups, followed by the colon and liver. This pattern was also observed in the wogonin treatment group compared to the control group. This was compared favorably to published data(Talbi et al., 2014). Nevertheless, it is shown that the highest amount of MPA was accumulating both in the colon and small intestine. The concentrations of MPA were highest in the Colon, followed by the small intestine and the liver. The concentration of MPAG measured was highest in the small intestine, the colon, and finally, the liver. This

coincides with the individual measurement of the treatment drug in the tissues where the concentration was checked, and it was observed that the concentrations of MPA and MPAG in wogonin and chrysin were the highest. The liver's concentration of these two compounds was significantly lower which entails the localized effects of the flavonoids MPA and MPAG.

4.2 Tissue concentration of Wogonin and Chrysin

Chrysin and wogonin concentrations were highest in the small intestine, followed by the colon, and low in the liver. This confirms what other publications claim about the local bioavailability of flavonoids(AL-Ishaq et al., 2021b)

Wogonin has a low systemic bioavailability. Additional investigation revealed that this bioavailability might be related to wogonin's low solubility and quick in vivo interaction with glucuronic acid (Zhu et al., 2016). The results also revealed that it has a high bioavailability locally in the stomach.

Nevertheless, Chrysin, with its low water solubility, quick metabolism facilitated by UGTs and SULT, and effective excretion via efflux transporters, including BCRP and MRP2 as the main factors contributing to its low systemic bioavailability reported in-vitro (Gao et al., 2021), in this study, Chrysin has a very robust localized and increased bioavailability profile in the gut lumen which confirmed what had been earlier studied. Furthermore, because of enterohepatic recycling, hepatocytes secrete chrysin-glucuronide and chrysin-sulfate conjugates into the bile that enters the intestine. There, the conjugates can be quickly digested by the intestinal microbial beta-glucuronidase to liberate chrysin. (G. Yang et al., 2017)

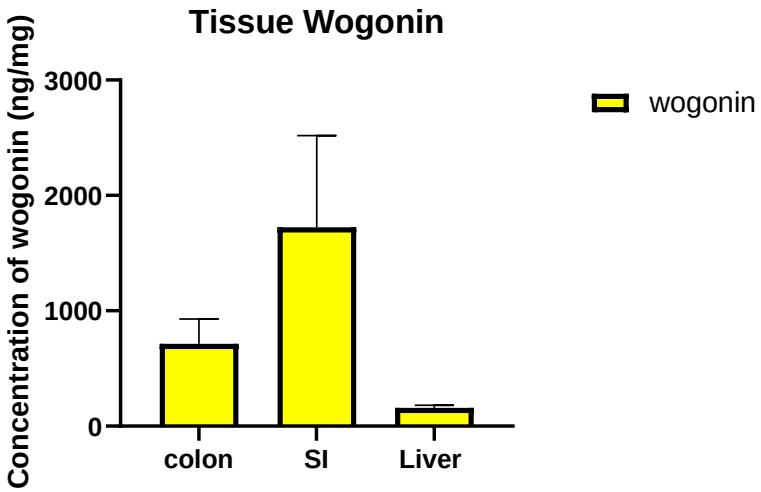

Figure 16. The concentration of Wogonin in the liver, small intestine, and colon. Data were expressed as mean ± SD

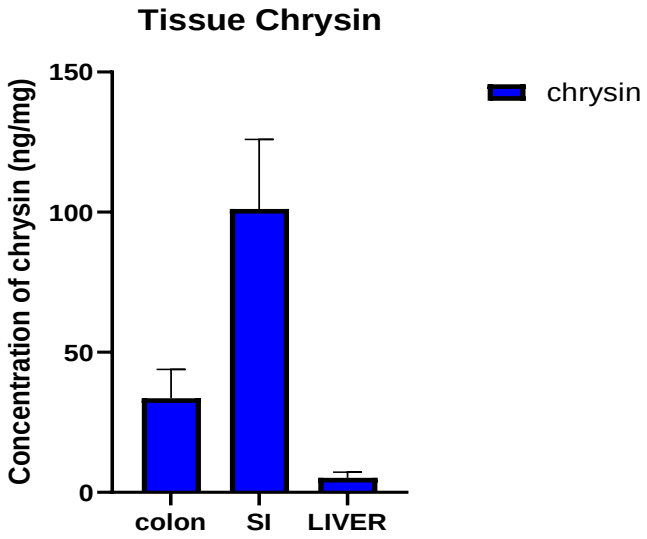

Figure 17. The concentration of Chrysin in the liver, small intestine, and colon. Data were expressed as mean ± SD

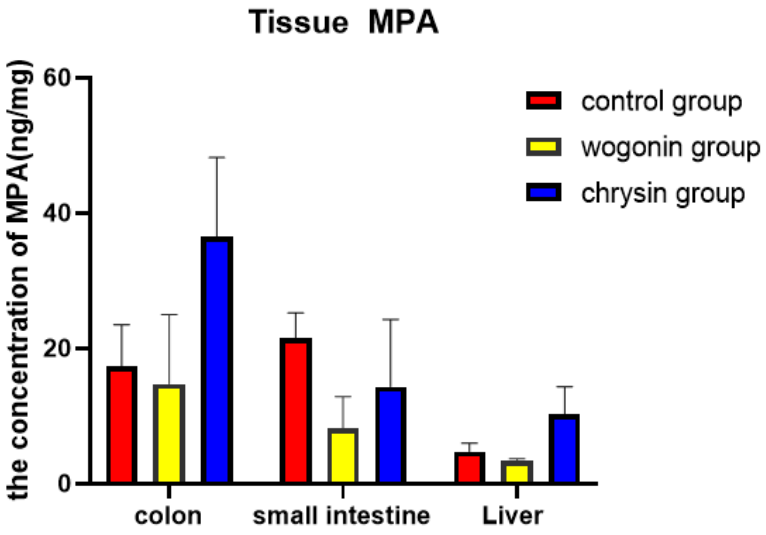

Figure 18. The concentrations of MPA show the distribution in the colon, small intestine, and liver for both the treatment and the control group. Data were expressed as mean ± SD

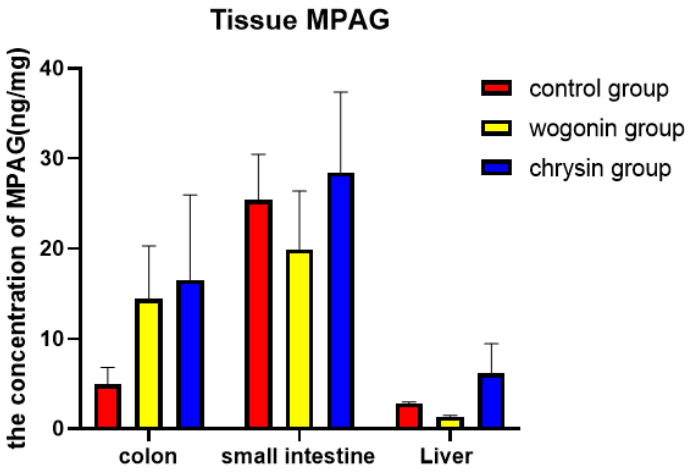

Figure 19. The concentrations of MPAG show the distribution in the colon, Small Intestine, and liver for both the treatment and the control group. Data were expressed as mean ± SD

4.2.1 Plasma Concentration-time Profile:

Pharmacokinetic profile for MPA

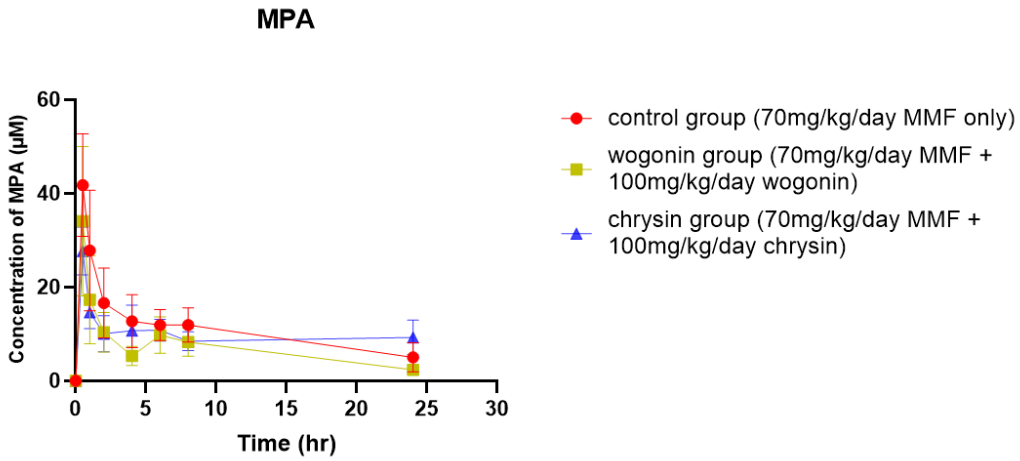

Figure 20. The PK profile of MPA of the Treatment batch dosed with MMF 70mg/kg/day along with 100mg/kg/day of Wogonin and Chrysin compared with the control group (MMF 70mg/kg/day only)

Pharmacokinetic profile for MPAG

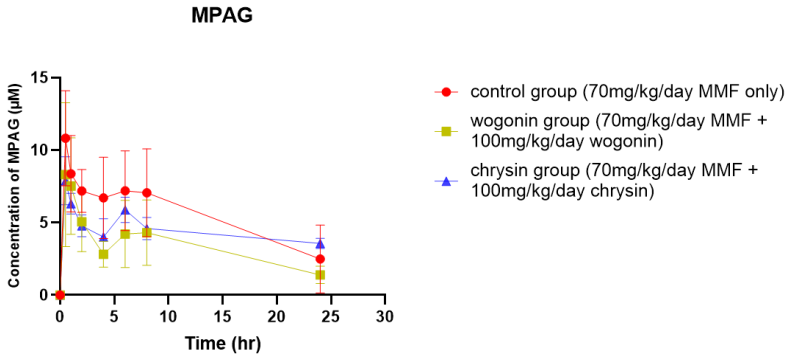

Figure 21. The PK profile of MPAG of the Treatment batch dosed with MMF 70mg/kg/day along with 100mg/kg/day of Wogonin and Chrysin compared with the control group (MMF 70mg/kg/day only)

According to Table 2 below, the lower the Tmax, the faster a drug gets absorbed into the system or local area. The group with MMF + Wogonin and MMF + Chrysin had the lowest Tmax overall, whereas the Control group with a Tmax of 4 suggests the formulation was not favored compared to others. This delay in the onset of activity might rather increase its toxicity since it would have still been in the active form. Also, the control group had the highest Cmax, which correlates with efficacy. However, this does not often necessarily translate to the anticipated better efficacy when considering other factors. It is very clear that the addition of the flavonoids, Wogonin and Chrysin greatly lowers the Cmax of MMF, hence this suggest that the combination of potentially reduce gastrointestinal adverse effects. We also observed the AUC, which represents the overall drug exposure. The higher the AUC, the greater the drug exposure. According to the data, although the addition of wogonin to MMF reduced the AUC of MPA, it is still moderate, and, with the moderate clearance when combined with wogonin, it shows that there is a moderate clearance minimizing duration potential toxic levels of MMF alone; however, this cannot on its own justify the activity.

Table 1. MPA PK data for treatment groups dosed 100mg

Parameters	**MMF Only**	**MMF + Wogonin**	**MMF + Chrysin**
Tmax (h)	0.50 ± 0.00	0.50 ± 0.00	0.5 ± 0.00
Cmax (µM)	41.76 ± 12.25	39.55 ± 14.98	26.40 ± 2.12
AUC0~t (hr*µM)	245.08 ± 89.89	165.50 ± 25.55	200.09 ± 57.22
MRT (h)	8.36 ± 3.39	8.00 ± 1.34	9.92 ± 0.33
T1/2 (h)	15.16 ± 7.29	8.43 ± 2.81	26.39 ± 8.42
CL mg/(hr*µM)/kg	0.23 ± 0.18	0.37 ± 0.08	0.17±0.02

Vz mg/(μM)/kg	3.96 ± 1.78	4.41 ± 1.08	6.47 ± 2.75

Both Chrysin and Wogonin groups showed low Cmax compared to MMF, indicating potential modulation of MMF concentration by adding the flavonoid compounds. Also, the wogonin group shows a very low AUC compared to the control and wogonin groups, potentially suggesting an elimination or metabolism effect. The mean residence time (MRT) indicates no significant difference. Meanwhile, the Wogonin and Crysin groups show reduced half-life compared to MMF, which means faster elimination. This was further buttressed by the clearance observed.

It was also observed that though wogonin did not alter the volume of distribution, the chrysin group showed higher distribution than the control.

Generally, the result shows that adding Chrysin and Wogonin to MMF enhances clearance and reduces its half-life, suggesting faster elimination kinetics and possible lowering of systemic exposure. Chrysin modulated the Pharmacokinetics of MMF, leading to decreased systemic exposure without significantly affecting Tmax or MRT, and this could mean that it is better to have a combination of chrysin and wogonin in further studies since wogonin clearly shows its efficacy more than Chrysin.

Table 2. MPAG Pharmacokinetic profile

Parameters	**MMF Only**	**MMF + Wogonin**	**MMF + Chrysin**
Tmax (h)	0.50 ± 0.00	0.50 ± 0.00	0.5 ± 0.00
Cmax (μM)	11.67 ± 4.15	7.48 ± 4.98	8.09 ±1.31
AUC0~t (hr*μM)	78.29 ± 26.18	63.45 ± 17.14	101.21 ± 3.99
MRT (h)	7.79 ± 3.43	9.54 ± 1.77	10.88 ± 0.59

T1/2 (h)	31.15 ± 26.84	16.17 ± 9.75	28.18 ± 5.57
CL mg/(hr*μM)/kg	0.37 ± 0.28	0.83 ± 0.39	0.29 ± 0.03
Vz mg/(μM)/kg	10.37 ± 5.47	16.10 ± 4.16	11.97 ± 1.07

Wogonin increases MPAG's Tmax quickly when added to MMF, indicating a possible influence on MPAG's metabolism or excretion. This is in tandem with our earlier hypothesis. The increased Cmax for MPAG in the MMF-only group may suggest a more significant conversion of MPA to MPAG in this group. Furthermore, the combination groups' (MMF + Wogonin and MMF + Chrysin) decreased AUC0~t for MPAG may indicate changes in MPAG metabolism and surely enterohepatic recycling prevention cannot be ruled out. The higher T1/2 for MPAG in the MMF + Chrysin group suggests that MPAG has been present in the body longer than in the other groups, maybe due to different elimination mechanisms. The MMF + Chrysin group's decreased MPAG clearance suggests that the MPAG metabolism may have changed or the clearance pathways may have been inhibited.

However, the impact of wogonin and chrysin seems to vary with dose and age, as the magnitude and consistency of the differences defer between the two, which could also be attributed to the age difference in the two sets of animals used as both metabolisms seem to differ based on rate. The 100mg/kg/day exhibits less pronounced changes in Pharmacokinetics parameters than the 50mg/kg, indicating potentially different pharmacokinetic interactions and might be dose dependent.

CHAPTER 5

SUMMARY AND CONCLUSION

Mycophenolate mofetil is utilized to prevent or treat allograft rejection after solid-organ transplantation. However, it causes dose-limiting gastrointestinal toxicity; the enzyme known as bacterial beta-glucuronidase bio transforms the conjugated form MPAG back to the active metabolite MPA in the gastrointestinal system, causing diarrhea in a process called enterohepatic recycling, which poses a significant challenge. Similarly, the carboxylesterase enzyme contributes to the continuous exposure of the active medication in the small intestine by hydrolyzing MMF to the MPA in the intestine.

Our first aim of this study was to evaluate the efficacy of Wogonin and Chrysin in alleviating Mycophenolate mofetil-induced diarrhea. We achieved this by utilizing our lab's pre-existing animal diarrhea model, which involves feeding the animals regular diets and administering Mycophenolate Mofetil doses at 70 mg/kg. This served as our control throughout the experiment. In the treatment group, the animals were orally treated with 100mg/kg of wogonin and repeated for chrysin in addition to 70mg/kg of MMF. Next, to determine whether the flavonoids were beneficial in reducing diarrhea, we assessed the clinical parameters and illness index scores, including body weight, diarrhea score, and survival analysis.

Following the experimental procedure, the oral flavonoids wogonin and chrysin were pretreated for two days before co-administration with Mycophenolate Mofetil for the remaining seven days.

This made it clear that preventing Mycophenolate Mofetil-induced diarrhea could effectively manage this severe diarrhea side effect. In contrast to the control group (Mycophenolate Mofetil alone), whose animals experienced severe diarrhea and body weight loss, the oral flavonoids treatment group underwent extensive diarrhea attenuation and maintained body weight under these experimental conditions. If GI toxicity is minimized, the risk of graft loss may be reversed by returning to the pre-reduction MMF dose. (Bunnapradist & Ambühl, 2008)

The rat mainly exhibited grade one to three diarrhea, indicating that the wogonin and chrysin therapy was not harmful when the therapeutic effects of oral flavonoids and mycophenolate mofetil were assessed. On the other hand, animals exposed to large amounts of mycophenolate mofetil had grade 4 diarrhea, and some even passed away. This supports the assertion that there is no evidence of gastrointestinal toxicity in preclinical species (like rats) when exposed to naturally occurring flavonoids(Galati & O'Brien, 2004).

Apart from preventing weight loss, the survival plot revealed that the wogonin treatment group significantly lessened the chances of death, thereby improving survival. This effect can possibly be due to non-erosion of the mucosa of the small and colon intestines in the presence of wogonin and chrysin. This was confirmed in an earlier study of the effects of wogonin and chrysin on the release of inflammatory cytokines. (Meng et al., 2017b)

This study demonstrated no significant differences in the PK profiles of the oral flavonoids wogonin, Chrysin, and Mycophenolate Mofetil-only group regarding the plasma drug concentration-time profile. With these results, we hypothesize that wogonin

and chrysin, when administered with Mycophenolate Mofetil, can effectively reduce the GI toxicity without impacting their Pharmacokinetic profile of mycophenolate mofetil.

REFERENCES

Abraham, B. P., & Sellin, J. H. (2019). Drug-Induced Diarrhea. In *Evidence-based Gastroenterology and Hepatology 4e* (pp. 208–224). Wiley. https://doi.org/10.1002/9781119211419.ch14

Aguiar, L. M., Geraldi, M. V., Betim Cazarin, C. B., & Maróstica Junior, M. R. (2019). Functional Food Consumption and Its Physiological Effects. In *Bioactive Compounds* (pp. 205–225). Elsevier. https://doi.org/10.1016/B978-0-12-814774-0.00011-6

Ahmed, R., Hassan, Z., Haseeb, A., Masood, A., & Ali, I. (2021). Multiple Adverse Drug Reactions to Calcineurin Inhibitors in a Renal Transplant Patient. *Uro*, *1*(3), 180–186. https://doi.org/10.3390/uro1030018

AL-Ishaq, R. K., Liskova, A., Kubatka, P., & Büsselberg, D. (2021a). Enzymatic Metabolism of Flavonoids by Gut Microbiota and Its Impact on Gastrointestinal Cancer. *Cancers*, *13*(16), 3934. https://doi.org/10.3390/cancers13163934

AL-Ishaq, R. K., Liskova, A., Kubatka, P., & Büsselberg, D. (2021b). Enzymatic Metabolism of Flavonoids by Gut Microbiota and Its Impact on Gastrointestinal Cancer. *Cancers*, *13*(16), 3934. https://doi.org/10.3390/cancers13163934

Allison, A. (2005). Mechanisms of action of mycophenolate mofetil. *Lupus*, *14*(3_suppl), 2–8. https://doi.org/10.1191/0961203305LU2109OA

Angarone, M., & Ison, M. G. (2015). Diarrhea in solid organ transplant recipients. *Current Opinion in Infectious Diseases*, *28*(4), 308–316.

https://doi.org/10.1097/QCO.0000000000000172

Angarone, M., & Snydman, D. R. (2019). Diagnosis and management of diarrhea in solid-organ transplant recipients: Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. *Clinical Transplantation*, *33*(9). https://doi.org/10.1111/ctr.13550

Arslan, H., Inci, E. K., Azap, O. K., Karakayali, H., Torgay, A., & Haberal, M. (2007). Etiologic agents of diarrhea in solid organ recipients. *Transplant Infectious Disease*, *9*(4), 270–275. https://doi.org/10.1111/j.1399-3062.2007.00237.x

Asrani, S. K., Leise, M. D., West, C. P., Murad, M. H., Pedersen, R. A., Erwin, P. J., Tian, J., Wiesner, R. H., & Kim, W. R. (2010). Use of sirolimus in liver transplant recipients with renal insufficiency: A systematic review and meta-analysis. *Hepatology*, *52*(4), 1360–1370. https://doi.org/10.1002/hep.23835

Bowalgaha, K., & Miners, J. O. (2001). The glucuronidation of mycophenolic acid by human liver, kidney and jejunum microsomes. *British Journal of Clinical Pharmacology*, *52*(5), 605–609. https://doi.org/10.1046/j.0306-5251.2001.01487.x

Bullingham, R., Monroe, S., Nicholls, A., & Hale, M. (1996). Pharmacokinetics and Bioavailability of Mycophenolate Mofetil in Healthy Subjects after Single-Dose Oral and Intravenous Administration. *The Journal of Clinical Pharmacology*, *36*(4), 315–324. https://doi.org/10.1002/j.1552-4604.1996.tb04207.x

Bunnapradist, S., & Ambühl, P. M. (2008). Impact of gastrointestinal-related side effects on mycophenolate mofetil dosing and potential therapeutic strategies. *Clinical Transplantation*, *22*(6), 815–821. https://doi.org/10.1111/j.1399-0012.2008.00892.x

Bunnapradist, S., Neri, L., Wong, W., Lentine, K. L., Burroughs, T. E., Pinsky, B. W.,

Takemoto, S. K., & Schnitzler, M. A. (2008). Incidence and Risk Factors for Diarrhea Following Kidney Transplantation and Association With Graft Loss and Mortality. *American Journal of Kidney Diseases*, *51*(3), 478–486. https://doi.org/10.1053/j.ajkd.2007.11.013

Cardet, J.-C., & Boyce, J. A. (2013). Addition of mycophenolate mofetil to tacrolimus is associated with decreases in food-specific IgE levels in a pediatric patient with liver transplantation-associated food allergy. *The Journal of Allergy and Clinical Immunology: In Practice*, *1*(1), 104–106. https://doi.org/10.1016/j.jaip.2012.08.001

Chassany, O., Michaux, A., & Bergmann, J. F. (2000). Drug-Induced Diarrhoea. *Drug Safety*, *22*(1), 53–72. https://doi.org/10.2165/00002018-200022010-00005

Chen, S., Wang, X., Cheng, Y., Gao, H., & Chen, X. (2023). A Review of Classification, Biosynthesis, Biological Activities and Potential Applications of Flavonoids. *Molecules*, *28*(13), 4982. https://doi.org/10.3390/molecules28134982

Cossart, A. R., Cottrell, W. N., Campbell, S. B., Isbel, N. M., & Staatz, C. E. (2019). Characterizing the pharmacokinetics and pharmacodynamics of immunosuppressant medicines and patient outcomes in elderly renal transplant patients. *Translational Andrology and Urology*, *8*(S2), S198–S213. https://doi.org/10.21037/tau.2018.10.16

Cushnie, T. P. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, *26*(5), 343–356. https://doi.org/10.1016/j.ijantimicag.2005.09.002

Dalle, I. J., Maes, B. D., Geboes, K. P., Lemahieu, W., & Geboes, K. (2005). Crohn's-like changes in the colon due to mycophenolate? *Colorectal Disease*, *7*(1), 27–34. https://doi.org/10.1111/j.1463-1318.2004.00695.x

Deng, Y., Zhang, Z., Yang, H., Wang, J., Feng, L., Su, Y., & Xu, D. (2022). Mycophenolic Acid Induces the Intestinal Epithelial Barrier Damage through Mitochondrial ROS. *Oxidative Medicine and Cellular Longevity*, *2022*, 1–18. https://doi.org/10.1155/2022/4195699

Dhakal, P., Gami, R., Appiah, A., Giri, S., Sayles, H., & Bhatt, V. (2017). Clinical Features and Outcomes of Mycophenolate Mofetil-induced Diarrhea: A Systematic Review. *Journal of Advances in Medicine and Medical Research*, *24*(6), 1–9. https://doi.org/10.9734/JAMMR/2017/37412

Docampo, M., Olubu, A., Wang, X., Pasinetti, G., & Dixon, R. A. (2017). Glucuronidated Flavonoids in Neurological Protection: Structural Analysis and Approaches for Chemical and Biological Synthesis. *Journal of Agricultural and Food Chemistry*, *65*(35), 7607–7623. https://doi.org/10.1021/acs.jafc.7b02633

Donovan, R., Eisen, H., & Ali, O. (2021). *Antiproliferatives and Transplantation* (pp. 39–52). https://doi.org/10.1007/164_2021_556

Ekberg, H., Tedesco-Silva, H., Demirbas, A., Vítko, Š., Nashan, B., Gürkan, A., Margreiter, R., Hugo, C., Grinyó, J. M., Frei, U., Vanrenterghem, Y., Daloze, P., & Halloran, P. F. (2007). Reduced Exposure to Calcineurin Inhibitors in Renal Transplantation. *New England Journal of Medicine*, *357*(25), 2562–2575. https://doi.org/10.1056/NEJMoa067411

Elnaggar, M. S., Ibrahim, N., Elissawy, A. M., Anwar, A., Ibrahim, M. A. A., & Ebada, S. S. (2024). Cytotoxic and antimicrobial mycophenolic acid derivatives from an endophytic fungus Penicillium sp. MNP–HS–2 associated with Macrozamia communis. *Phytochemistry*, *217*, 113901.

https://doi.org/10.1016/j.phytochem.2023.113901

FAN, X., & SELLIN, J. H. (2009). Review article: small intestinal bacterial overgrowth, bile acid malabsorption and gluten intolerance as possible causes of chronic watery diarrhoea. *Alimentary Pharmacology & Therapeutics*, *29*(10), 1069–1077. https://doi.org/10.1111/j.1365-2036.2009.03970.x

Farooqi, R., Kamal, A., & Burke, C. (2020). Mycophenolate-induced Colitis: A Case Report with Focused Review of Literature. *Cureus*, *12*(1), e6774. https://doi.org/10.7759/cureus.6774

Fletcher, J. T., Nankivell, B. J., & Alexander, S. I. (2009). Chronic allograft nephropathy. *Pediatric Nephrology*, *24*(8), 1465–1471. https://doi.org/10.1007/s00467-008-0869-z

Fukushima, K., Lappin, M., Legare, M., & Veir, J. (2021). A retrospective study of adverse effects of mycophenolate mofetil administration to dogs with immune-mediated disease. *Journal of Veterinary Internal Medicine*, *35*(5), 2215–2221. https://doi.org/10.1111/jvim.16209

Galati, G., & O'Brien, P. J. (2004). Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. *Free Radical Biology and Medicine*, *37*(3), 287–303. https://doi.org/10.1016/j.freeradbiomed.2004.04.034

Gao, S., Siddiqui, N., Etim, I., Du, T., Zhang, Y., & Liang, D. (2021). Developing nutritional component chrysin as a therapeutic agent: Bioavailability and pharmacokinetics consideration, and ADME mechanisms. *Biomedicine & Pharmacotherapy*, *142*, 112080. https://doi.org/10.1016/j.biopha.2021.112080

Gerdin, B., & Svensjö, E. (1983). Inhibitory effect of the flavonoid O-(beta-hydroxyethyl)-rutoside on increased microvascular permeability induced by various agents in rat skin. *International Journal of Microcirculation, Clinical and Experimental*, *2*(1), 39–46.

Ginsburg, P. M., & Thuluvath, P. J. (2005). Diarrhea in liver transplant recipients: Etiology and management. *Liver Transplantation*, *11*(8), 881–890. https://doi.org/10.1002/lt.20500

Guerra, G., Ciancio, G., Gaynor, J. J., Zarak, A., Brown, R., Hanson, L., Sageshima, J., Roth, D., Chen, L., Kupin, W., Tueros, L., Ruiz, P., Livingstone, A. S., & Burke, G. W. (2011). Randomized Trial of Immunosuppressive Regimens in Renal Transplantation. *Journal of the American Society of Nephrology*, *22*(9), 1758–1768. https://doi.org/10.1681/ASN.2011010006

Guerrant, R. L., Van Gilder, T., Steiner, T. S., Thielman, N. M., Slutsker, L., Tauxe, R. V., Hennessy, T., Griffin, P. M., DuPont, H., Bradley Sack, R., Tarr, P., Neill, M., Nachamkin, I., Reller, L. B., Osterholm, M. T., Bennish, M. L., & Pickering, L. K. (2001). Practice Guidelines for the Management of Infectious Diarrhea. *Clinical Infectious Diseases*, *32*(3), 331–351. https://doi.org/10.1086/318514

Javdan, B., Lopez, J. G., Chankhamjon, P., Lee, Y.-C. J., Hull, R., Wu, Q., Wang, X., Chatterjee, S., & Donia, M. S. (2020). Personalized Mapping of Drug Metabolism by the Human Gut Microbiome. *Cell*, *181*(7), 1661-1679.e22. https://doi.org/10.1016/j.cell.2020.05.001

Jeong, H., & Kaplan, B. (2007). Therapeutic Monitoring of Mycophenolate Mofetil. *Clinical Journal of the American Society of Nephrology*, *2*(1), 184–191.

https://doi.org/10.2215/CJN.02860806

Jia, Y., Wang, R., Li, L., Zhang, Y., Li, J., Wang, J., Wang, X., Qi, G., Rong, R., Xu, M., & Zhu, T. (2018). Sites of gastrointestinal lesion induced by mycophenolate mofetil: a comparison with enteric-coated mycophenolate sodium in rats. *BMC Pharmacology & Toxicology*, *19*(1), 39. https://doi.org/10.1186/s40360-018-0234-1

Khan, S., Khan, T., & Shah, A. J. (2018). Total phenolic and flavonoid contents and antihypertensive effect of the crude extract and fractions of Calamintha vulgaris. *Phytomedicine*, *47*, 174–183. https://doi.org/10.1016/j.phymed.2018.04.046

Klawitter, J., Nashan, B., & Christians, U. (2015). Everolimus and sirolimus in transplantation-related but different. *Expert Opinion on Drug Safety*, *14*(7), 1055–1070. https://doi.org/10.1517/14740338.2015.1040388

Kumar, S., & Pandey, A. K. (2013). Chemistry and Biological Activities of Flavonoids: An Overview. *The Scientific World Journal*, *2013*, 1–16. https://doi.org/10.1155/2013/162750

Manito, N., Delgado, J. F., Crespo-Leiro, M. G., González-Vílchez, F., Almenar, L., Arizón, J. M., Díaz, B., Fernández-Yáñez, J., Mirabet, S., Palomo, J., Rodríguez Lambert, J. L., Roig, E., & Segovia, J. (2010). Clinical recommendations for the use of everolimus in heart transplantation. *Transplantation Reviews*, *24*(3), 129–142. https://doi.org/10.1016/j.trre.2010.01.005

Matas, A. J., Smith, J. M., Skeans, M. A., Thompson, B., Gustafson, S. K., Stewart, D. E., Cherikh, W. S., Wainright, J. L., Boyle, G., Snyder, J. J., Israni, A. K., & Kasiske, B. L. (2015). OPTN/SRTR 2013 Annual Data Report: Kidney. *American Journal of Transplantation*, *15*, 1–34. https://doi.org/10.1111/ajt.13195

Meng, X., Fang, S., Zhang, Z., Wang, Y., You, C., Zhang, J., & Yan, H. (2017a). Preventive effect of chrysin on experimental autoimmune uveitis triggered by injection of human IRBP peptide 1-20 in mice. *Cellular & Molecular Immunology*, *14*(8), 702–711. https://doi.org/10.1038/cmi.2015.107

Meng, X., Fang, S., Zhang, Z., Wang, Y., You, C., Zhang, J., & Yan, H. (2017b). Preventive effect of chrysin on experimental autoimmune uveitis triggered by injection of human IRBP peptide 1–20 in mice. *Cellular & Molecular Immunology*, *14*(8), 702–711. https://doi.org/10.1038/cmi.2015.107

Menter, A., Cordoro, K. M., Davis, D. M. R., Kroshinsky, D., Paller, A. S., Armstrong, A. W., Connor, C., Elewski, B. E., Gelfand, J. M., Gordon, K. B., Gottlieb, A. B., Kaplan, D. H., Kavanaugh, A., Kiselica, M., Kivelevitch, D., Korman, N. J., Lebwohl, M., Leonardi, C. L., Lichten, J., … Elmets, C. A. (2020). Joint American Academy of Dermatology–National Psoriasis Foundation guidelines of care for the management and treatment of psoriasis in pediatric patients. *Journal of the American Academy of Dermatology*, *82*(1), 161–201. https://doi.org/10.1016/j.jaad.2019.08.049

Merali, Z., Ross, S., & Paré, G. (2014). The pharmacogenetics of carboxylesterases: *CES1* and *CES2* genetic variants and their clinical effect. *Drug Metabolism and Drug Interactions*, *29*(3), 143–151. https://doi.org/10.1515/dmdi-2014-0009

Milani, N., Parrott, N., Ortiz Franyuti, D., Godoy, P., Galetin, A., Gertz, M., & Fowler, S. (2022). Application of a gut–liver-on-a-chip device and mechanistic modelling to the quantitative *in vitro* pharmacokinetic study of mycophenolate mofetil. *Lab on a Chip*, *22*(15), 2853–2868. https://doi.org/10.1039/D2LC00276K

Mizumoto, C., Kanda, J., Ichinohe, T., Ishikawa, T., Matsui, M., Kadowaki, N., Kondo, T., Imada, K., Hishizawa, M., Kawabata, H., Nishikori, M., Yamashita, K., Takaori-Kondo, A., Hori, T., & Uchiyama, T. (2009). Mycophenolate mofetil combined with tacrolimus and minidose methotrexate after unrelated donor bone marrow transplantation with reduced-intensity conditioning. *International Journal of Hematology*, *89*(4), 538–545. https://doi.org/10.1007/s12185-009-0306-5

Nakamura, M., Ogawa, N., Shalabi, A., Maley, W. R., & Burdick, J. F. (2001). Positive effect on T-cell regulatory apoptosis by mycophenolate mofetil. *Clinical Transplantation*, *15*(s6), 36–40. https://doi.org/10.1034/j.1399-0012.2001.00006.x

Papadimitriou, J. C., Cangro, C. B., Lustberg, A., Khaled, A., Nogueira, J., Wiland, A., Ramos, E., Klassen, D. K., & Drachenberg, C. B. (2003). Histologic Features of Mycophenolate Mofetil-Related Colitis: A Graft-Versus-Host Disease-Like Pattern. *International Journal of Surgical Pathology*, *11*(4), 295–302. https://doi.org/10.1177/106689690301100406

Parfitt, J. R., Jayakumar, S., & Driman, D. K. (2008a). Mycophenolate Mofetil-related Gastrointestinal Mucosal Injury: Variable Injury Patterns, Including Graft-versus-Host Disease-like Changes. *American Journal of Surgical Pathology*, *32*(9), 1367–1372. https://doi.org/10.1097/PAS.0b013e31816bf3fe

Parfitt, J. R., Jayakumar, S., & Driman, D. K. (2008b). Mycophenolate Mofetil-related Gastrointestinal Mucosal Injury: Variable Injury Patterns, Including Graft-versus-Host Disease-like Changes. *American Journal of Surgical Pathology*, *32*(9), 1367–1372. https://doi.org/10.1097/PAS.0b013e31816bf3fe

Park, W. Y., Paek, J. H., Jin, K., Park, S. B., & Han, S. (2019). Clinical Significance of

Mycophenolate Mofetil Withdrawal in Kidney Transplant Recipients. *Transplantation Proceedings*, *51*(8), 2633–2636. https://doi.org/10.1016/j.transproceed.2019.03.061

Peddi, V. R., Wiseman, A., Chavin, K., & Slakey, D. (2013). Review of combination therapy with mTOR inhibitors and tacrolimus minimization after transplantation. *Transplantation Reviews*, *27*(4), 97–107. https://doi.org/10.1016/j.trre.2013.06.001

Ponticelli, C., & Glassock, R. J. (2019). Prevention of complications from use of conventional immunosuppressants: a critical review. *Journal of Nephrology*, *32*(6), 851–870. https://doi.org/10.1007/s40620-019-00602-5

Saqr, A., Carlson, B., Staley, C., Rashidi, A., Al-Kofahi, M., Kaiser, T., Holtan, S., MacMillan, M., Young, J.-A., Jurdi, N. El, Weisdorf, D., Khoruts, A., & Jacobson, P. A. (2022). Reduced Enterohepatic Recirculation of Mycophenolate and Lower Blood Concentrations Are Associated with the Stool Bacterial Microbiome after Hematopoietic Cell Transplantation. *Transplantation and Cellular Therapy*, *28*(7), 372.e1-372.e9. https://doi.org/10.1016/j.jtct.2022.04.018

Satoh, T., Taylor, P., Bosron, W. F., Sanghani, S. P., Hosokawa, M., & Du, B. N. La. (2002). Current Progress on Esterases: From Molecular Structure to Function. *Drug Metabolism and Disposition*, *30*(5), 488–493. https://doi.org/10.1124/dmd.30.5.488

Sekmek, S., Yildirim, T., Koc, N. S., Onal, C., Jabrayilov, J., Yilmaz, R., Arici, M., Altun, B., & Erdem, Y. (2021). Efficacy and Safety of Switching to Azathioprine for Mycophenolate-Induced Diarrhea in Renal Transplant Recipients. *Transplantation Proceedings*, *53*(6), 1951–1956. https://doi.org/10.1016/j.transproceed.2021.06.020

Smith, L. (2003). Corticosteroids in Solid Organ Transplantation: Update and Review of

the Literature. *Journal of Pharmacy Practice*, *16*(6), 380–387. https://doi.org/10.1177/0897190003259838

Soefje, S. A., Karnad, A., & Brenner, A. J. (2011). Common toxicities of mammalian target of rapamycin inhibitors. *Targeted Oncology*, *6*(2), 125–129. https://doi.org/10.1007/s11523-011-0174-9

Staatz, C. E., & Tett, S. E. (2007). Clinical Pharmacokinetics and Pharmacodynamics of Mycophenolate in Solid Organ Transplant Recipients. *Clinical Pharmacokinetics*, *46*(1), 13–58. https://doi.org/10.2165/00003088-200746010-00002

Stumpf, J., Siepmann, T., Schwöbel, J., Glombig, G., Paliege, A., Steglich, A., Gembardt, F., Kessel, F., Kröger, H., Arndt, P., Sradnick, J., Frank, K., Klimova, A., Mauer, R., Tonn, T., & Hugo, C. (2022). MMF/MPA Is the Main Mediator of a Delayed Humoral Response With Reduced Antibody Decline in Kidney Transplant Recipients After SARS-CoV-2 mRNA Vaccination. *Frontiers in Medicine*, *9*. https://doi.org/10.3389/fmed.2022.928542

Sutherland, A. I., IJzermans, J. N. M., Forsythe, J. L. R., & Dor, F. J. M. F. (2016). Kidney and liver transplantation in the elderly. *British Journal of Surgery*, *103*(2), e62–e72. https://doi.org/10.1002/bjs.10064

Talbi, A., Zhao, D., Liu, Q., Li, J., Fan, A., Yang, W., Han, X., & Chen, X. (2014). Pharmacokinetics, Tissue Distribution, Excretion and Plasma Protein Binding Studies of Wogonin in Rats. *Molecules*, *19*(5), 5538–5549. https://doi.org/10.3390/molecules19055538

Thielman, N. M., & Guerrant, R. L. (2004). Acute Infectious Diarrhea. *New England Journal of Medicine*, *350*(1), 38–47. https://doi.org/10.1056/NEJMcp031534

van Gelder, T., & Hesselink, D. A. (2015). Mycophenolate revisited. *Transplant International*, *28*(5), 508–515. https://doi.org/10.1111/tri.12554

van Gelder, T., Hilbrands, L. B., Vanrenterghem, Y., Weimar, W., de Fijter, J. W., Squifflet, J. P., Hen??, R. J., Verpooten, G. A., Navarro, M. T., Hale, M. D., & Nicholls, A. J. (1999). A RANDOMIZED DOUBLE-BLIND, MULTICENTER PLASMA CONCENTRATION CONTROLLED STUDY OF THE SAFETY AND EFFICACY OF ORAL MYCOPHENOLATE MOFETIL FOR THE PREVENTION OF ACUTE REJECTION AFTER KIDNEY TRANSPLANTATION1. *Transplantation*, *68*(2), 261–266. https://doi.org/10.1097/00007890-199907270-00018

Verri, W. A., Vicentini, F. T. M. C., Baracat, M. M., Georgetti, S. R., Cardoso, R. D. R., Cunha, T. M., Ferreira, S. H., Cunha, F. Q., Fonseca, M. J. V., & Casagrande, R. (2012). *Flavonoids as Anti-Inflammatory and Analgesic Drugs: Mechanisms of Action and Perspectives in the Development of Pharmaceutical Forms* (pp. 297–330). https://doi.org/10.1016/B978-0-444-53836-9.00026-8

VICENTE, O., & BOSCAIU, M. (2018). Flavonoids: Antioxidant Compounds for Plant Defence... and for a Healthy Human Diet. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *46*(1), 14–21. https://doi.org/10.15835/nbha46110992

Wieland, E., Shipkova, M., Schellhaas, U., Schütz, E., Niedmann, P. D., Armstrong, V. W., & Oellerich, M. (2000). Induction of cytokine release by the acyl glucuronide of mycophenolic acid: a link to side effects? *Clinical Biochemistry*, *33*(2), 107–113. https://doi.org/10.1016/S0009-9120(99)00101-0

Xia, B., Zhou, Q., Zheng, Z., Ye, L., Hu, M., & Liu, Z. (2012). A Novel Local Recycling

Mechanism That Enhances Enteric Bioavailability of Flavonoids and Prolongs Their Residence Time in the Gut. *Molecular Pharmaceutics*, *9*(11), 3246–3258. https://doi.org/10.1021/mp300315d

Xiao, H., Ma, S., Qi, H., Li, X., Wang, Y., & Yin, C. (2016). A scoring system for assessing the severity of acute diarrhea of adult patients. *World Journal of Emergency Medicine*, *7*(2), 130. https://doi.org/10.5847/wjem.j.1920-8642.2016.02.008

Xu, X., Hou, Y., Yin, X., Bao, L., Tang, A., Song, L., Li, F., Tsang, S., Wu, K., Wu, H., He, W., Zeng, L., Xing, M., Wu, R., Jiang, H., Liu, X., Cao, D., Guo, G., Hu, X., … Wang, J. (2012). Single-Cell Exome Sequencing Reveals Single-Nucleotide Mutation Characteristics of a Kidney Tumor. *Cell*, *148*(5), 886–895. https://doi.org/10.1016/j.cell.2012.02.025

Yang, G., Ge, S., Singh, R., Basu, S., Shatzer, K., Zen, M., Liu, J., Tu, Y., Zhang, C., Wei, J., Shi, J., Zhu, L., Liu, Z., Wang, Y., Gao, S., & Hu, M. (2017). Glucuronidation: driving factors and their impact on glucuronide disposition. *Drug Metabolism Reviews*, *49*(2), 105–138. https://doi.org/10.1080/03602532.2017.1293682

Yang, J. C.-H., Reguart, N., Barinoff, J., Köhler, J., Uttenreuther-Fischer, M., Stammberger, U., O'Brien, D., Wolf, J., & Cohen, E. E. (2013). Diarrhea associated with afatinib: an oral ErbB family blocker. *Expert Review of Anticancer Therapy*, *13*(6), 729–736. https://doi.org/10.1586/era.13.31

Yu, C., Seidel, K., Nash, R. A., Deeg, H. J., Sandmaier, B. M., Barsoukov, A., Santos, E., & Storb, R. (1998). Synergism Between Mycophenolate Mofetil and Cyclosporine

in Preventing Graft-Versus-Host Disease Among Lethally Irradiated Dogs Given DLA-Nonidentical Unrelated Marrow Grafts. *Blood*, *91*(7), 2581–2587. https://doi.org/10.1182/blood.V91.7.2581

Zhao, L., Yuan, X., Wang, J., Feng, Y., Ji, F., Li, Z., & Bian, J. (2019). A review on flavones targeting serine/threonine protein kinases for potential anticancer drugs. *Bioorganic & Medicinal Chemistry*, *27*(5), 677–685. https://doi.org/10.1016/j.bmc.2019.01.027

Zimmermann, M., Zimmermann-Kogadeeva, M., Wegmann, R., & Goodman, A. L. (2019). Mapping human microbiome drug metabolism by gut bacteria and their genes. *Nature*, *570*(7762), 462–467. https://doi.org/10.1038/s41586-019-1291-3

Zimmermann-Kogadeeva, M., Zimmermann, M., & Goodman, A. L. (2020). Insights from pharmacokinetic models of host-microbiome drug metabolism. *Gut Microbes*, *11*(3), 587–596. https://doi.org/10.1080/19490976.2019.1667724