PROCEEDINGS

The 46th of the Pharmacological and Therapeutic Society of Thailand Annual Meeting

Innovative and Sustainable Pharmacological Approaches for Combating Non-Communicable Diseases (NCDs)

15-17 May 2025

The Bed Vacation Hotel

Songkhla

THE PHARMACOLOGICAL AND THERAPEUTIC SOCIETY OF THAILAND

FACULTY OF SCIENCE
PRINCE OF SONGKLA UNIVERSITY

This publication includes abstracts of the invited speakers and poster abstracts and papers presented at the 46th Annual Meeting of the Pharmacological and Therapeutic Society of Thailand under the theme "Innovative and Sustainable Pharmacological Approaches for Combating Non-Communicable Diseases (NCDs)". The conference was held at the Bed Vacation Hotel, Songkhla from May 15-17, 2025, organized by Pharmacology Program, Division of Health and Applied Science, Faculty of Science, Prince of Songkla University, Songkhla, Thailand.

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Welcome Message from the Chair of Organizing Committee

Welcome Address

Distinguished Guests, Esteemed Colleagues, and Friends,

It is with great honor and enthusiasm that I welcome you all to the **46th Annual Meeting of the Pharmacological and Therapeutic Society of Thailand**, a gathering of exceptional minds dedicated to advancing the field of pharmacological science. Our theme this year, **"Innovative and Sustainable Pharmacological Approaches for Combating Non-Communicable Diseases (NCDs)"**, underscores our shared commitment to addressing some of the most pressing health challenges of our time.

As we embark on this journey together, we are excited to explore **updates and trends in cardiovascular pharmacology**, delve into the evolving landscape of **Type 2 diabetes treatments**, and examine the therapeutic potential of **Kratom**. Moreover, we will highlight the transformative possibilities of **precision medicine**, an approach that epitomizes the future of tailored healthcare.

This event represents not only an opportunity to exchange knowledge and insights but also a chance to forge collaborations that can drive meaningful change. Together, we will discuss breakthroughs, challenge established paradigms, and pave the way for sustainable and impactful pharmacological solutions.

Your dedication to innovation and your passion for improving human health are the cornerstones of progress. I encourage you to engage wholeheartedly in the presentations, discussions, and workshops over the course of this meeting. Let us harness the collective brilliance in this room to envision and create a healthier, more equitable future.

Once again, welcome to this exciting and pivotal conference. Let us make the 46th Annual Meeting of the Pharmacological and Therapeutic Society of Thailand a landmark in our shared journey toward transformative healthcare.

Thank you. Let the dialogue begin!

Wandee Udomuksorn

Assistant Professor Wandee Udomuksorn, Ph.D.
Chair of the Organizing Committee

Welcome Message

Rattima Jeenapongsa

President, Pharmacological and Therapeutic Society of Thailand

Dear Colleagues, Guests, and Friends,

It is a great honor to welcome you to the 46th the Pharmacological and Therapeutic Society of Thailand Annual Meeting held on 15 to 17 May 2025, co-hosted by Faculty of Science, Prince of Songkla University. This year, we convene under the theme "Innovative and Sustainable Pharmacological Approaches for Combating Non-Communicable Diseases (NCDs)," a theme that underscores our collective mission to drive transformative research and therapeutic advancements in addressing global health challenges.

As we navigate an era of rapid scientific progress, the burden of non-communicable diseases (NCDs) continues to rise, necessitating a multi-faceted, evidence-based, and sustainable approach to their prevention and treatment. This meeting serves as a platform for leading researchers, clinicians, and policymakers to share knowledge, collaborate on cutting-edge therapies, and pave the way for future breakthroughs in pharmacology and therapeutics.

This year's program features an outstanding lineup of symposia that reflect the breadth and depth of innovation in pharmacological sciences. We will explore novel drug development strategies, precision medicine, phytopharmacology, cardiovascular and metabolic diseases, oncology, and the transformative role of data science in healthcare.

The discussions at this meeting will shape the future of pharmacology and therapeutics by addressing both current challenges and emerging opportunities in NCD management. Through scientific exchange, we hope to foster stronger collaborations, inspire new research directions, and promote sustainable and patient-centered approaches in pharmacotherapy.

Warm Regards,
Associate Professor Rattima Jeenapongsa, Ph.D.
President, The Pharmacological and Therapeutic Society of Thailand (PTST)

Day 1 (Thu 15 May 2025)	
Time	**Sessions**
08.00 - 08.30	**Registration**
08.30 - 09.00	**Welcome Ceremony** Professor Anchana Prathep, Ph.D. Dean of Faculty of Science, Prince of Songkla University Assistant Professor Wandee Udomuksorn, Ph.D. Chair of the Organizing Committee (PTST2025) Associate Professor Rattima Jeenapongsa, Ph.D. President of the Pharmacological and Therapeutic Society of Thailand *Moderated by Assistant Professor Sucharat Tungsukruthai, Ph.D.*
09.00 - 10.00	**[Eng] Chiravat Sadavongvivad Memorial Lecture** **"Mechanism Underlying Aortic Degeneration and Introduction of a Clinical Trial Using Tricaprin and Beta caryophyllene"** Professor Nobuhiro Zaima, Ph.D. Department of Applied Biological Chemistry / Graduate School of Agriculture / Agricultural Technology and Innovation Research Institute, Kindai University, Japan *Moderated by Associate Professor Supita Tanasawet, Ph.D.*
10.00 - 10.15	**Technical Seminar I** **Building Interactive Class: The Application of Teaching Tools** CHEN YAYI, Chengdu Techman Software Co., Ltd
10.15 - 10.30	Morning Coffee break (In room)
10.30 - 11.30	**[Eng] Plenary session I: Novel targets in addressing cardiovascular problems: from synthesis to potential drug development** [Online] **"Effects of Methotrexate on Blood Pressure and Wave Reflection in Rheumatoid Arthritis: A Randomized Controlled Trial"** Dr. Sara Tommasi and Dr. Julie-Ann Hulin Flinders University, Australia *Moderated by Dr. Warit Ruanglertboon*
11.30 - 12.00	**[Thai] Ouay Ketusingh Honorary Award Ceremony and Award Lecture** • Professor Boonjua Thoranin, Ph.D., Department of Pharmacology, Faculty of Medicine Siriraj Hospital, Mahidol University • Associate Professor Mingkwan Na takuathung, Ph.D., Department of Pharmacology, Faculty of Medicine, Chiang Mai University *Moderated by Professor Uraiwan Panich, M.D., Ph.D.*
12.00 - 13.00	**Lunch**
13.00 – 13.45	**[Thai] Symposium I: Update and trends in CVS pharmacology** **Update Drug Treatment Regimen for CVS Disease** **"Guideline-Directed Medical Therapy in Heart Failure"** Assistant Professor Thanapon Nilmoje, M.D. Division of Internal Medicine, Faculty of Medicine, Prince of Songkla University *Moderated by Associate Professor Wanida Sukketsiri, Ph.D.*
13.45 - 14.30	**[Thai] Symposium II: Update kratom situation and pharmacology trends** **"Kratom-Drug Interactions: A Pharmacokinetic Perspective on the Complexities of its Use in Clinical Practice"** Associate Professor Verawan Uchaipichat, Ph.D. Department of Clinical Pharmacy, Faculty of Pharmaceutical Science, Khon Kaen University *Moderated by Assistant Professor Somchai Sriwiriyajan, Ph.D.*
14.30 - 15.15	**[Thai] Symposium II: Update kratom situation and pharmacology trends** **"Studies of Kratom Plant Effects on Central Nervous System Using Digital Platforms"** Associate Professor Ekkasit Kumarnsit, Ph.D. Division of Health and Applied Science, Faculty of Science, Prince of Songkla University *Moderated by Assistant Professor Somchai Sriwiriyajan, Ph.D.*
15.15 - 15.30	**Technical Seminar II** **"Exploration of Exosome Profiling with Flow Cytometry and Immunoassays: Key Tools for Early-Stage Drug Discovery and Biomarker Identification"**

	Atchara Chothiphirat, Senior Technical Application Specialist for Cell & Cellular Analysis Products, Gibthai Co., Ltd.
15.30 - 15.45	Afternoon refreshments
15.45 – 17.30	**Poster Session**
17.30 – 18.30	**Annual Member Meeting (2nd Call)** The Pharmacological and Therapeutic Society of Thailand
18.30 - 20.30	**Social dinner and Honoring Ceremony**

Day 2 (Fri 16 May 2025)	
Time	**Sessions**
08.30 - 09.00	**Registration**
09.00 - 10.00	**[Thai] Plenary session II: Update biology & pathology and treatments regimen for NCDs** **"Update and Trends in DM Type II Treatment"** Assistant Professor Noppadol Kietsiriroje, M.D. (Endocrine and metabolism), Ph.D. Division of Internal Medicine, Faculty of Medicine, Prince of Songkla University *Moderated by Assistant Professor Supattra Limsuwannachote, Ph.D.*
10.00 - 10.15	**Technical Seminar III** **"Novel Biobank Potential with Smart Freezers"** Pornwarin Ouncharoen, Scion Associated Limited Partnership
10.15 - 10.30	Morning Coffee break (In room)
10.30 – 12.00	**[Thai] Plenary session III: Frontiers of prevention and non- pharmacological interventions for NCD** **"How to do DM Remission?"** Dr. Thanasak Yimkerd, M.D. (Endocrine and metabolism) Diet doctor Thailand & Diet doctor clinic *Moderated by Assistant Professor Wandee Udomuksorn, Ph.D.*
12.00 - 13.00	**Lunch**
13.00 - 13.45	**[Thai] Symposium III: Update and trends in cancer therapy & precision medicine in cancer** **"Targeting Cancer Stem Cells; Insights into Molecular Pharmacology, Structure-Activity Relationship of Natural Product-Derived Compounds"** Professor Pithi Chanvorachote, Ph.D. Department of Pharmacology & Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University *Moderated by Assistant Professor Sucharat Tungsukruthai, Ph.D.*
13.45-14.30	**[Thai] Symposium III: Update and trends in cancer therapy & Precision medicine in cancer** **"Preclinical Approach Using the Patient-Derived Cell Lines with Integrative Genomic Profiling and Network Pharmacology in Precision Cancer Care"** Associate Professor Sarinya Kongpetch, Ph.D. Department of Pharmacology, Faculty of Medicine, Khon Kaen University *Moderated by Assistant Professor Sucharat Tungsukruthai, Ph.D.*
14.30 - 14.45	**Technical Seminar IV** **"Accelerating Pharmacological Research Through Technology Integration"** Mr. Aas Mohammad, Sales manager (Asia Pacific), Fortis Trading Co.,Ltd.
14.45 – 15.00	Afternoon refreshments
15.00 – 15.45	**[Thai] Symposium IV: Update and trends in pharmacogenomics** **"Update on Pharmacogenomics of Severe Cutaneous Adverse Reactions: From Bench to Clinical Implementations"** Professor Wichittra Tassaneeyakul, Ph.D. Department of Pharmacology, Faculty of Medicine, Khon Kaen University *Moderated by Dr. Warit Ruanglertboon*
15.45 – 16.15	**[Thai] Symposium V: Young pharmacologists forum** **"Health and Clinical Data Science: Using Modern Tools to Fine-Tuned Clinical Pharmacotherapeutic Practice"** Dr. Warit Ruanglertboon Pharmacology Program, Division of Health Science Faculty of Science, Prince of Songkla University *Moderated by Dr. Kornsuda Thipart*
16.15 – 16.45	**Poster Award Announcement and Closing Ceremony**

Day 3 (Sat 17 May 2025)	
08.30 – 09.00	**Registration**
09.00 – 10.30	Knowledge Sharing for Pharmacology Teaching
10.30 – 10.45	Morning refreshments
10.45 – 12.00	Knowledge Sharing for Pharmacology Teaching

Chiravat Sadavongvivad's Memorial Lecture

Mechanism Underlying Aortic Degeneration and Introduction of a Clinical Trial using Tricaprin and Beta caryophyllene

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Abstract

Blood flow supplies essential compounds for cells which is basic building blocks of our body. Collapse of the circulatory system will have serious problems on vital functions and cardiovascular disease is the leading cause of death worldwide. Maintaining of healthy blood vessel can be effective strategy for preventing the cardiovascular diseases. Among the initiators of the cardiovascular diseases, aortic degeneration is widely observed in several cases including atherosclerosis and aneurysms. We reported the aortic degeneration can be caused by several factors such as genetic mutation (NEJM 2008), focal aortic hypoperfusion (Plos One 2015), smoking (Biotech. Histochem. 2017), unbalanced diets (J. Vasc. Res. 2018, Adipocyte 2021), menopause (Sci. Rep. 2019), and malnutrition (Adipocyte 2021). Here, I introduce our research data and some experimental animal models to investigate the relationship between aortic degeneration and the contributing factors.

As second topic, I would like to introduce potential strategies to establish prevention methods for cardiovascular diseases. We found aortic degeneration and cardiovascular diseases can be attenuated by functional food factors such as fish oil (Sci. Rep. 2016, Food & Function 2021, 2022, J. Lipid Res. 2022), beta-caryophyllene (Biomed. Pharmacother. 2022), tricaprin (Biomed. Pharmacother. 2022, Nature Cardiovasc. Res. 2025), turmerone (Fitoterapia 2025). Here, I introduce basic research and some clinical studies and devices for preventing aortic degeneration.

Because aortic degeneration can be caused by multiple factors, availability of several options for prevention is desirable to establish prevention methods for cardiovascular diseases. I would like to discuss about potential strategies for preventing cardiovascular diseases in this session.

Keywords: Aortic degeneration, animal models, aneurysm, tricaprin, beta-caryophyllene

Effects of Methotrexate on Blood Pressure and Wave Reflection in Rheumatoid Arthritis: A Randomized Controlled Trial

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Abstract

Observational studies suggest that methotrexate (MTX) treatment is independently associated with lower cardiovascular risk in rheumatoid arthritis (RA) patients when compared to other disease-modifying antirheumatic drugs (DMARDs). Such effect seems to be primarily associated with a reduction in blood pressure (BP), however a cause-effect relationship between MTX and BP lowering remains to be demonstrated.

We conducted a controlled comparative study of treatment-naïve newly diagnosed RA patients commenced on subcutaneous methotrexate (Group 1, n=31, age 57±15 years, 65% females, Disease Activity Score-28 – C-reactive protein, DAS28-CRP=4.7±1.2) or the DMARD comparator sulfasalazine (Group 2, n=31, 54±17 years, 61% females, DAS28-CRP=5.0±0.8). Clinic systolic (SBP, primary study endpoint) and diastolic BP (DBP), augmentation index (AIx, a marker of arterial stiffness) and DAS28 were measured at baseline and after one and six months of treatment (ClinicalTrials.gov: NCT03254589). After 1 month, there was no between-group difference in the changes in SBP and DBP (delta= -4.9mmg; 95%CI=-10.6, 0.7, p=0.08 and delta=-3.1 mmHg; 95%CI=-7.1, 1.0, p=0.14, respectively). There was a significant reduction in DAS28CRP (delta=-0.67; 95%CI=-1.24, -0.10, p=0.03), but not DAS28ESR (delta=-0.46; 95%CI=-1.15, 0.24, p=0.19), for group 1 vs. group 2. After 6 months, group 1 had a significant reduction in SBP versus group 2 (delta=-7.4 mmHg; 95%CI=-14.0, -0.8, p=0.03), without significant between-group differences in changes in DBP, AIx, or DAS28. There was a significant effect of single nucleotide polymorphisms (SNPs) rs1801133 (methyl tetrahydrofolate reductase) and rs2231142 (ATP-binding cassette subfamily G member 2) on BP changes during methotrexate treatment.

This study provides the first evidence that MTX causes a significant reduction in SBP at 6 months in RA patients. This effect is not mediated by changes in wave reflection or disease activity. Further research is warranted to identify the mechanisms involved in the MTX-induced BP-lowering effects and whether such effects mediate the cardiovascular risk reduction during MTX treatment.

Update Drug Treatment Regimen for CVS Disease "Guideline-Directed Medical Therapy in Heart Failure"

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Abstract

Heart failure (HF) remains a major cause of morbidity and mortality and represents the common endpoint of diverse cardiovascular insults. It is classified by left ventricular ejection fraction (LVEF) into three phenotypes as HFrEF (reduced): LVEF < 40%, HFmrEF (mildly reduced): LVEF 40 – 49%, HFpEF (preserved): LVEF ⩾ 50%

Guideline-Directed Medical Therapy (GDMT) aims to reduce sudden cardiac death and HF hospitalizations across all LVEF categories. Four classes of medications form the foundation of GDMT: RAS inhibitors (ARNI / ACEI / ARB), Beta-blockers, Mineralocorticoid receptor antagonists, SGLT2 inhibitors.

In HFrEF, all four drug classes carry a Class I recommendation. Until recently, HFmrEF and HFpEF lacked comparable evidence; however, the 2023 European Society of Cardiology and Thai heart failure guidelines elevated SGLT2 inhibitors to Class I in HFmr and HFpEF, solidifying their role across the EF spectrum.

Originally developed for glucose lowering in type 2 diabetes, SGLT2 inhibitors demonstrated unexpected cardiovascular benefits after rigorous outcome trials became mandatory post-2008. The landmark EMPA-REG OUTCOME trial first revealed reductions in HF events. Subsequent studies—DECLARE-TIMI 58, DAPA-HF, and EMPEROR-Reduced—confirmed their efficacy in HFrEF. More recent trials, EMPEROR-Preserved and DELIVER, extended these benefits to HFpEF, addressing a long-standing therapeutic gap.

In summary, contemporary HF management hinges on early initiation of the four GDMT pillars, with SGLT2 inhibitors now recognized as a transformative therapy that improves outcomes across all LVEF-defined HF phenotypes.

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Kratom-Drug Interactions: A Pharmacokinetic Perspective on the Complexities of its Use in Clinical Practice

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Abstract

Kratom (*Mitragyna speciosa*), a traditional herbal product widely used for its stimulant and analgesic properties, has gained increasing global attention due to its potential for therapeutic use and challenging drug interaction profile. Despite its traditional use, Kratom has the potential for addiction, leading to differing legal classifications across countries. In Thailand, Kratom was banned under the Narcotics Act since 1943 but was recently delisted from Schedule 5, making its use legal. This regulatory change, along with increased accessibility, may contribute to a rise in herb-drug interaction cases due to more widespread consumption. This presentation reviews the pharmacokinetic aspects of Kratom-drug interactions, focusing on the inhibitory effects of its key active alkaloids—mitragynine and 7-hydroxymitragynine—on major drug-metabolizing enzymes, particularly cytochrome P450s (CYPs) and UDP-glucuronosyltransferases (UGTs). Numerous studies have explored how its extract and active components affect various CYP isoforms, with the strongest inhibitory activity observed on CYP2D6 and CYP3A enzymes. Notably, potential interactions between Kratom and other drugs—likely due to the inhibition of CYP3A, CYP2D6, or P-glycoprotein—have been implicated in several fatal incidents. Investigations into UGT inhibition have been more limited, with earlier studies focusing on UGT1A1 and UGT2B7—where 7-hydroxymitragynine demonstrated significant inhibition and mitragynine showed only modest effects. Recent work from our laboratory expanded this understanding by evaluating the impact of these alkaloids on a broader panel of UGT isoforms. Results demonstrated varying degrees of inhibition on UGT activities, with the most potent effects observed for mitragynine on UGT1A3 and 7-hydroxymitragynine on UGT1A9. This presentation provides a pharmacokinetic perspective on Kratom's interaction potential, emphasizing the clinical importance of drug-metabolizing enzyme inhibition and the challenges involved in its co-administration with therapeutic drugs.

References

1. Tanna et al., 2023. Drug Metab Dispos 51:923-935.
2. Uchaipichat, 2025. Toxicol Rep 14:101951.

Keywords: Herb-drug interaction, drug-metabolizing enzyme, 7-hydroxymitragynine, kratom, mitragynine

Studies of Kratom Plant Effects on Central Nervous System Using Digital Platforms

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Abstract

The effects of bioactive compounds can be investigated in many different ways. Digital platforms have been invented and introduced to drug discovery and pharmaceutical sciences in purposes to make a disruptive change in scientific research. By using equipment that biosignals can be detected and digitized, the data can be recorded in digital platform. This is to process the data using basic or modern algorithm together with program coding. This would allow for the analysis of big data. In Thailand, the use of kratom plant has been highly controversial. It's important to determine kratom plant's effects on the central nervous system (CNS). Kratom plant extracts were administered to animal models. Animal behaviors and physiological changes including cardiac autonomous activity and electrical brain signals were digitally recorded and stored in PC for offline analysis. For example, Fast Fourier transform was used for frequency analysis of animal's brain waves. With the results analyzed, kratom plant extracts were clearly confirmed to attenuate severities of ethanol withdrawal, methamphetamine dependence and produce antidepressant-like activity. Modified computer program coding was also applied to analyze animal's brain waves to examine kratom's effect on sleep-wake pattern. Altogether, these data are useful in terms of drug discovery. This platform is ideal for high throughput processing. In particular, new bioactive compounds can be studied using digital platform in addition to that of conventional methods.

Keywords: Kratom plant, drug discovery, central nervous system, digital data, data processing

Update and Trends in DM Type II Treatment

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Abstract

Diabetes treatment before 2015 was full of limitations. There were few types of medications available, offering a certain level of effectiveness in reducing blood sugar but without proven benefits in reducing the risk of cardiovascular disease or slowing kidney deterioration. The side effects of these drugs included hypoglycaemia and weight gain. Attempts to lower blood sugar to the target range with these limited medications may result in a serious adverse outcome: an increased rate of cardiovascular death among patients. The arrival of two drug classes, SGLT2 inhibitors and GLP-1 receptor agonists, marked a turning point. For the first time in the history of diabetes treatment, there were medications that could reduce cardiovascular disease events and slow kidney deterioration in diabetic patients. Importantly, these benefits were not solely due to blood sugar reduction but resulted from the drugs' mechanisms of action through other metabolic factors, including significant weight loss. This shifted the paradigm of diabetes care from focusing solely on blood sugar control to reducing the risk of cardiovascular and kidney diseases, or cardio-kidney-metabolic (CKM) syndrome. Furthermore, the remarkable weight loss efficacy of GLP-1 receptor agonists led to the development of new weight loss drugs, potentially addressing the root cause of diabetes and CKM-related conditions, which is obesity,

Keywords: SGLT2 inhibitor, GLP-1 receptor agonist, type 2 diabetes, obesity, cardio-kidney-metabolic syndrome

How to do DM Remission?

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Abstract

โรคเบาหวานชนิดที่ 2 เป็นโรคในกลุ่มโรคเรื้อรังที่เรียกว่า โรคไม่ติดต่อเรื้อรัง (NCDs) ในมุมมองที่ผ่านมาโรคนี้ถูกอธิบายว่าเกิดจากการดื้ออินซูลิน (Insulin Resistance) เป็นโรคที่รักษาไม่หาย เป้าหมายในการรักษาจึงโฟกัสอยู่ที่การควบคุมระดับน้ำตาลในเลือดให้ใกล้เคียงกับปกติ โดยการควบคุมอาหาร ออกกำลังกายและการใช้ยาควบคุมระดับน้ำตาลในเลือดรวมถึงการใช้อินซูลิน เนื่องจากโรคนี้มีความสัมพันธ์กับภาวะน้ำหนักเกินและโรคอ้วนจึงมีการโฟกัสการใช้ยาที่จดทะเบียนเพื่อลดน้ำหนักในกลุ่ม GLP-1 agonist โดยตั้งเป้าว่าการลดน้ำหนักจะทำให้ควบคุมระดับน้ำตาลในเลือดได้ดีขึ้นด้วย อย่างไรก็ตาม ตลอดเวลาที่ผ่านมาเราจะพบว่าแม้มีการพัฒนายาลดน้ำตาลในเลือดมาตลอดก็ไม่สามารถลดภาวะแทรกซ้อนของผู้ป่วยได้ ข้อแนะนำด้านโภชนาการที่เน้นอาหารจำกัดแคลอรีและไขมันต่ำโดยเน้นคาร์โบไฮเดรต 50-55% ของพลังงานที่รับประทานต่อวันรวมถึงการรณรงค์การออกกำลังกายก็ดูจะชะลออุบัติการณ์ของโรคอ้วนและโรคเบาหวานชนิดที่ 2 ไม่ได้ รวมถึงอุบัติการณ์การเกิดโรคในกลุ่มที่อายุไม่มากเริ่มเกิดสูงขึ้น ตลอดเวลาที่การรักษาเบาหวานชนิดที่ 2 มุ่งไปที่การควบคุมระดับน้ำตาลในเลือดและเรามีความเข้าใจว่าข้อแนะนำทางด้านโภชนาการที่เราใช้กันมาตลอดถูกต้องอยู่แล้ว เมื่อ 12 ปีที่ผ่านมามีแพทย์ผู้ดูแลผู้ป่วยเบาหวานชนิดที่ 2 และนักวิชาการส่วนหนึ่งได้พยายามทำความเข้าใจผลของโภชนาการต่อการรักษาโรคอ้วนและเบาหวานชนิดที่ 2 โดยอธิบายผ่านการศึกษาระบบเผาผลาญของร่างกาย พบว่าการจำกัดปริมาณคาร์โบไฮเดรตในอาหารสามารถทำให้การลดน้ำหนักประสบผลสำเร็จง่ายขึ้น ในผู้ป่วยเบาหวานชนิดที่ 2 ที่จำกัดคาร์โบไฮเดรตสามารถควบคุมระดับน้ำตาลในเลือดได้ง่ายและเร็วขึ้นจนสามารถหยุดยาได้ที่เรียกว่า “Diabetes Remission” จำนวนผู้ป่วยเบาหวานชนิดที่ 2 ที่เข้าสู่ Diabetes Remission มากขึ้นเรื่อย ๆ จนกระทั่งสมาคมโรคเบาหวานอเมริกาและยุโรปได้สรุป Diabetes Remission Consensus Statement ขึ้นเมื่อปลายปี 2021 จะเห็นได้ว่าโรคในกลุ่มระบบเผาผลาญดังเช่น โรคอ้วนและโรคเบาหวานชนิดที่ 2 ความเข้าใจในเรื่องโภชนาการและระบบเผาผลาญของร่างกายมีความสำคัญเป็นอย่างมาก มุมมองในการแก้ปัญหากลุ่มอาการโรคเรื้อรังที่รักษาไม่หายอาจจะไม่ใช่เรื่องการใช้ยาแต่เพียงอย่างเดียว หากโรคเหล่านี้มีความเกี่ยวข้องในเชิงสาเหตุมาจากโภชนาการที่อาจจะไม่ใช่อย่างที่เราคิด นั่นอาจจะเป็นสาเหตุที่ทำให้เราแก้ปัญหาไม่ตรงจุด “เราไม่สามารถแก้ปัญหาโรคที่เกิดจากโภชนาการผิดได้โดยการใช้ยาเคมี” การเปลี่ยนมุมมองที่เรามองโรคกลุ่มนี้อาจจะเป็นทางออกใหม่ที่จะช่วย

แก้ปัญหาให้กับผู้ที่ป่วยไปแล้ว ป้องกันการเกิดผู้ป่วยรายใหม่ และช่วยให้ผู้ที่ให้บริการในด้านการแพทย์และสาธารณสุขทำงานได้อย่างมีความสุขรวมถึงลดภาระงานที่ล้นเกินลงไปได้

Keywords: โรคอ้วน, โรคเบาหวานชนิดที่ 2, Insulin resistance, Diabetes Remission

Targeting Cancer Stem Cells; Insights into Molecular Pharmacology, Structure-Activity Relationship of Natural Product-Derived Compounds

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Abstract

Cancer stem cells (CSCs) play a pivotal role in tumor drug resistance and metastasis, significantly contributing to cancer-related mortality. These cells possess the unique ability to self-renew and differentiate into various specialized cell types. Advances in understanding CSC-associated molecular mechanisms have led to the development of targeted therapeutic strategies, with growing evidence highlighting their signaling pathways as potential drug targets. Preclinical studies indicate that inhibiting CSC pathways can enhance the effectiveness of conventional treatments, prompting ongoing clinical trials for CSC-targeting inhibitors. Our research focuses on lung cancer, emphasizing the therapeutic potential of bioactive compounds derived from natural sources such as plants and marine organisms. Among our promising lead compounds are resveratrol, a polyphenol found in grapes, blueberries, and cranberries, and Renieramycin T, an alkaloid isolated from the blue sponge *Xestospongia* sp. To optimize drug efficacy, we employ chemical modifications to establish Structure-Activity Relationships (SAR), enabling the development of more potent derivatives. By integrating molecular pharmacology with computational modeling, our studies identify key CSC-associated targets, including Protein Kinase B/mTOR and β-Catenin, while also predicting compound-protein interactions. Given the significant role of CSCs in tumor progression and their association with poor patient prognosis, discovering novel therapeutic agents targeting CSC pathways holds great promise for improving cancer treatment outcomes.

Keywords: Cancer stem cells, natural sources, structure-activity relationship

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Preclinical Approach Using the Patient-Derived Cell Lines with Integrative Genomic Profiling and Network Pharmacology in Precision Cancer Care

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Abstract

Cancer cell lines serve as a powerful tool for defining the mechanism of cancer growth and metastasis, as well as for developing therapeutic interventions to attack cancer vulnerabilities. Notably, genomic profiling of diverse tumor types has dramatically enhanced the ability to identify oncogenic alterations that can be targeted for therapy. Here, we showed the model of cholangiocarcinoma (CCA), a biliary tract carcinoma with complex and very heterogeneous malignancy. We established CCA cell lines that were derived from CCA patients in Srinagarind Hospital. Those established CCA cell lines with unique characters may serve as valuable tools for better developing new therapeutics for the patients. The CCA cell lines presented many targetable mutations with preclinical and clinical evidence. At least five actionable mutations (*ARID1A, BRCA1, PIK3CA, KRAS*, and *ATR* mutations) were identified. Then, we utilized synthetic lethality-based therapy to explore the sensitivity of poly (ADP-ribose) polymerase inhibitors (PARPi) in CCA cell lines that harbored DNA damage repair (DDR) deficiency. Analyzing publicly available databases for potential alterations in epigenetic and DDR genes in CCA patient samples revealed mutations in multiple genes, indicating their possible sensitivity to PARPi. We found that CCA cells with a high accumulation of mutations in epigenetic and other DDR-related genes demonstrated greater sensitivity to PARPi. Moreover, we identified flavonoids with anti-PARP activity by integrating molecular docking, transcriptomic analysis, and network pharmacology. These preclinical approaches could provide potential benefits as a valuable tool for drug testing to develop precision practice in oncology.

Keywords: Network pharmacology, genomic profiling, precision cancer care, targetable mutations, DNA damage repair

Update on Pharmacogenomics of Severe Cutaneous Adverse Reactions: From Bench to Clinical Implementations

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Abstract

Severe cutaneous adverse reactions (SCARs) are a group of adverse reactions resulting from drug-induced T cell-mediated hypersensitivity, characterized by severe skin eruptions and varying degrees of systemic involvement. These reactions consist of several distinct disease entities, including Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), drug reaction with eosinophilia and systemic symptoms (DRESS), acute generalized exanthematous pustulosis (AGEP), and generalized bullous fixed drug eruption (GBFDE). SJS and TEN are characterized by widespread blistering cutaneous eruptions, atypical targets, and extensive mucosal erosions. DRESS is a multisystem hypersensitivity reaction with a diffuse, infiltrative maculopapular eruption, visceral organ inflammation, and hematological abnormalities. AGEP and GBFDE are milder forms of SCARs. AGEP is characterized by the abrupt onset of numerous sterile pustules with painful erythema, classically following administration of antibiotics with a short latency period. GBFDE manifests as rapid onset of widespread erythematous patches and frequently recurs at the same sites as previous occurrences.

The top five causative drugs of SCARs in Thailand include allopurinol, co-trimoxazole, phenytoin, carbamazepine, and amoxicillin. Several factors have been identified as risks for these severe adverse drug reactions, including genetic factors, drug-related factors, patient-related factors, and environmental factors. Among them, genetic variants of the human leukocyte antigens (HLA) and drug-metabolizing enzymes have been extensively studied. *HLA-B*15:02* is associated with carbamazepine-induced SJS/TEN, *HLA-B*58:01* with allopurinol-induced SCARs, *HLA-B*57:01* with abacavir-induced SCARs, and *CYP2C9*3* with phenytoin-induced SCARs. To date, HLA variants associated with SCARs induced by co-trimoxazole, dapsone, phenytoin, and beta-lactam antibiotics have also been reported. Updated information about the risks of SCARs, the discoveries of these HLA markers, as well as clinical implementation of the HLA genetic tests will be discussed in the meeting.

Key words: Pharmacogenomic, drug-induced severe cutaneous adverse drug reaction, HLA testing

Health and Clinical Data Science: Using Modern Tools to Fine-Tuned Clinical Pharmacotherapeutic Practice

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Abstract

Pharmacology has significantly evolved by integrating multiple dimensions of science and technology, becoming essential to both basic scientific understanding and clinical practice. Recent advancements in artificial intelligence, data science, and statistical learning have prominently featured in pharmacological applications, particularly in managing real-world clinical data. These advancements effectively address common data-quality challenges, such as incomplete entries and data entry errors, which are prevalent in unstructured hospital-level data. This presentation demonstrates practical applications of data science in pharmacology through illustrative examples, including landmark Cox proportional hazard analysis, linear mixed-effects modelling, and support vector machines. Emphasis is placed on essential data wrangling skills, such as regex-based cleaning techniques for drug names, ensuring high-quality data analysis.

The first case study involves patients treated with sorafenib, analysed using actual clinical trial data. Real-world challenges, particularly dose adjustments due to adverse events, led approximately 40% of patients to experience dose interruptions and 30% to require dose reductions. Using landmark Cox proportional hazard analysis, the association between early dose adjustments and patient outcomes was examined. An extension of this analysis employed alternative data-wrangling methods to explore whether concurrent use of proton pump inhibitors with sorafenib impacted cancer survival outcomes. The third case study utilised the same dataset but employed machine learning techniques, including random forest and decision tree analyses. Integrating these machine learning methods with traditional statistical analyses demonstrated robust capabilities in enhancing predictive accuracy and clinical decision-making.

The presentation concluded with an analysis of a local hospital dataset, focusing on the efficacy of the long-established drug allopurinol over a decade of clinical use. Extensive data manipulation provided insights into the drug's performance, validated through linear mixed-effects models and predictive efficacy analyses using support vector machine techniques.

A01

A Predict Optimal Dosage Regimens of Photoactivated Curcumin in Bacterial-Resistant Infection Therapy: A Design of Extensive Release of Oral Dosage Form and Long-Acting Intramuscular Injection

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Abstract

Photoactivated curcumin is a promising antibiotic candidate for treating bacterial resistance, demonstrating a favorable safety and efficacy profile. However, current formulations of curcumin used in clinical trials have not succeeded in providing adequate drug concentrations for combating bacterial infections. Physiologically-based pharmacokinetic (PBPK) modeling is a bottom-up approach used to predict drug disposition. This method leverages existing knowledge from *in vitro* and *in vivo* studies, employing a mathematical framework that describes the anatomical, biological, and molecular processes involved in pharmacokinetics (PK). By integrating the relationship between drug exposure and efficacy/toxicity (pharmacodynamics – PD) into the PBPK model, it becomes possible to create a practical framework for simulating dose-finding strategies tailored to specific patient populations, such as the elderly, pediatrics, pregnant women, and obese individuals. In addition to predicting doses, PBPK modeling can aid in designing novel formulations and exploring alternative routes of administration for various clinical scenarios. When PBPK modeling is combined with well-defined PD modeling (for example, anti-infective activity) or PK/PD indices, it becomes a powerful tool for rationalizing dose-finding strategies for new antimicrobial candidates. The objective of this study was to develop comprehensive whole-body PBPK models to determine appropriate dosing regimens for photoactivated curcumin as an antimicrobial treatment for bacterial-resistant infections. We built a whole-body PBPK model using Simbiology® (a MATLAB® product) based on *in vitro* data and human physiological parameters. A virtual population of 100 patients (comprising 50 females and 50 males) was created through a Monte Carlo simulation, with participants aged between 18 and 60 years. The assumptions of our model included: 1) similarities in the pharmacokinetic and physicochemical properties between photoactivated curcumin and curcumin (with only minor changes after photoactivation), 2) blood-flow-limited characteristics for all organs (due to the hydrophobic nature of the compound), 3) instantaneous distribution of the compound into tissues (assumed to be well-stirred), 4) no absorption of the compound in the large intestine (indicating high absorption in the small intestine), and 5) no enterohepatic recirculation (with minimal curcumin present in feces). We validated our developed model against clinical data from a study of curcumin administered at oral doses of 4000, 6000, and 8000 mg, comparing pharmacokinetic parameter profiles and graphical data. This verified model was utilized to simulate novel dosing strategies for formulations of photoactivated curcumin. The results

showed that the once-daily and twice-daily oral dosing regimens for photoactivated curcumin were influenced by absorption rates and water solubility properties. Considering long-acting intramuscular dosing, a weekly dose of 1500 mg with a first-order release rate from the intramuscular depot (K_{im}) of 0.012/h, and a weekly dose of 1250 mg with a K_{im} of 0.015 resulted in adequate drug exposure. In conclusion, PBPK modeling serves as a valuable tool for optimizing dosing strategies of photoactivated curcumin under various clinical conditions. The weekly intramuscular injection may enhance patient compliance in diseases requiring long-term treatment while also reducing selective pressure and drug resistance.

Keywords: Photoactivated curcumin, controlled release formulation, physiologically-based pharmacokinetic model, bacterial-resistant infection.

A02

Hawm Gra Dang Ngah Rice Extract Attenuates Oleic Acid-Induced Hepatic Steatosis in HepG2 Cells Through Modulation of mTOR/ERK Signaling

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Abstract

Fatty liver disease is characterized by the accumulation of fat, mainly in the form of triglycerides, within liver cells, leading to cellular injury and subsequent inflammation. Current primary treatments mainly focus on lifestyle changes, highlighting a need for direct pharmacological options. Hawm Gra Dang Ngah rice (HGR) is a red rice variety cultivated in Thailand's southern border region, yet its biological properties have not been thoroughly studied. Therefore, this research investigates the effects and mechanisms of action of HGR extract against oleic acid (OA)-induced hepatosteatosis in HepG2 cells. Intracellular reactive oxygen species (ROS) production and apoptosis was measured using a 2',7'-dichlorofluorescin-diacetate and Hoechst 33342 staining. Lipid accumulation in OA-induced HepG2 cells was measured using Oil Red O staining and a triglyceride kit. Protein and gene expression were evaluated using Western blot and real-time RT-qPCR, respectively. The results demonstrated that ROS in OA-induced HepG2 cells were reduced following exposure to 50 and 100 µg/mL HGR extract, similarly to the positive drugs metformin and atorvastatin. This reduction was achieved by upregulating the expression of nuclear respiratory factor 1 and nuclear factor erythroid 2-related factor 2. HGR extract decreased the number of apoptotic cells by suppressing the caspase-3 pathway. It also decreased hepatic inflammation by downregulating the mRNA expression of tumor necrosis factor-alpha, interleukin (IL)-1β, and IL-6, while upregulating the anti-inflammatory cytokine IL-10. Additionally, HGR extract and the positive drugs decreased lipid accumulation in OA-induced HepG2 cells by regulating key molecules involved in lipid metabolism, including CD36, peroxisome proliferator-activated receptor gamma (PPARγ), PPARα, carnitine palmitoyltransferase I, fatty acid synthase, and adipose triglyceride lipase. Additionally, HGR extract significantly inhibited the phosphorylation of the mTOR and ERK pathways, which contributes to decreased lipid accumulation and inflammation in hepatocytes. These findings suggest that HGR extract mitigates hepatosteatosis by decreasing inflammation, apoptosis, and lipid accumulation, highlighting its potential as a therapeutic agent for hepatosteatosis.

Keywords: Hepatic steatosis, reactive oxygen species, apoptosis, beta-oxidation, *Oryza sativa*

Therapeutic Potential of Astilbin in Nonalcoholic Fatty Liver Disease: Attenuation of Oxidative Stress, Inflammation, and Lipid Accumulation

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Abstract

Nonalcoholic fatty liver disease (NAFLD), driven by oxidative stress, inflammation, insulin resistance, and obesity, poses significant economic and public health challenges. Effective treatment is hindered by limited pharmacological options, demanding a multifaceted approach. Astilbin (ASB), a flavonoid glycoside derived from diverse botanical sources, shows promise with antioxidant, anti-inflammatory, and metabolic benefits. However, the underlying mechanisms in NAFLD remain to be elucidated. This study aimed to investigate the antioxidant and anti-inflammatory properties of ASB and to evaluate its therapeutic potential for ameliorating intracellular lipid accumulation in an experimental model of NAFLD. ASB cytotoxicity was evaluated in Raw 264.7 and HepG2 cells via MTT assay. Raw 264.7 cells were used to assess antioxidant and anti-inflammatory effects; superoxide (O_2^-) levels were quantified by chemiluminescence after phorbol myristate acetate (PMA) stimulation, and nitric oxide (NO) levels by Griess assay after lipopolysaccharide (LPS) stimulation. To mimic NAFLD conditions, palmitic acid (PA)-induced lipotoxic in HepG2 was conducted, lipid droplets were visualized using Oil-Red-O staining. Protective effects of ASB were assessed against these stimuli. All experiments were performed in triplicate. Statistical significance was determined using one-way ANOVA followed by Student-Newman-Keuls analysis ($p < 0.05$). ASB exhibited IC_{50} values of 755.3 ± 2.53 µM (HepG2) and 547.9± 2.35 µM (Raw 264.7); sublethal doses (≤ 200 µM) were used for further study. ASB suppressed PMA-induced O_2^- formation and prevented LPS-induced morphological changes and decrease NO levels in a dose-dependent manner. Furthermore, ASB (200 µM) reduced lipid droplets by 35% ($p < 0.002$), similar to metformin's 42% reduction ($p < 0.001$), countering the 74% increase seen with PA alone ($p < 0.001$). Given oxidative stress and inflammation are critical in NAFLD pathogenesis. ASB exhibits antioxidant and anti-inflammatory effects and reduces lipid accumulation. Thus, clinical research into ASB's application for NAFLD and related metabolic disorders is warranted.

Keywords: Non-alcoholic fatty liver disease (NAFLD), astilbin, steatosis, oxidative stress, inflammation, metabolic disorders

A04

Beta-Caryophyllene Reduces Oxidative Stress and Inflammation in Hydrogen Peroxide-Induced Human Dermal Fibroblasts

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Abstract

Skin aging is influenced by multiple factors, including inflammation and oxidative stress, which lead to structural and functional impairments. Beta-caryophyllene (BCP), a bicyclic sesquiterpene, exhibits pharmacological properties such as anti-inflammatory and antioxidant effects, which are crucial mechanisms in delaying skin aging. However, studies investigating the anti-aging effects of BCP remain limited. Therefore, this study aimed to explore the protective effects and underlying molecular mechanism of BCP against oxidative damage in human dermal fibroblasts (HDFs). MTT assays revealed that BCP (0–1000 μM) showed no cytotoxicity. Under hydrogen peroxide (H_2O_2) exposure, BCP significantly reduced reactive oxygen species (ROS) production, as demonstrated by DCFH-DA staining, and mitigated apoptosis, as evidenced by Hoechst 33342 staining. Mechanistically, BCP upregulated nuclear factor erythroid 2–related factor 2 (Nrf2) and heme oxygenase-1 (HO-1), thereby enhancing antioxidant defenses. Gene expression analysis revealed that BCP increased levels of *fibrillin-1, collagen type I, collagen type III*, and *elastin*, while reducing the expression of pro-inflammatory markers *interleukin (IL)-1β, IL-6*, and *tumor necrosis factor-alpha (TNF-α)*. Western blotting further demonstrated that BCP suppressed the mTOR, Akt, ERK, and p38 signaling pathways, which are associated with oxidative stress and inflammation. Additionally, BCP inhibited the expression of nuclear factor-kappa B (NF-κB). Collective, these findings suggest that BCP mitigates oxidative stress, apoptosis, and inflammation by inhibiting the ERK, p38 MAPK, mTOR, Akt, and NF-κB pathways, supporting the potential development of BCP as an active ingredient in anti-aging skincare formulations.

Keywords: Beta-caryophyllene, oxidative stress, inflammation, human dermal fibroblast, skin aging

A05

Passion Fruit Seed and Roselle Calyx Extracts Suppress Lipid Accumulation and Inflammation in Oleic Acid-Induced Hepatosteatosis in HepG2 Cells

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Abstract

Fatty liver disease is characterized by excessive triglyceride accumulation within liver cells, leading to cellular injury and inflammation. In Thailand, passion fruit (*Passiflora edulis*) and roselle (*Hibiscus sabdariffa* L.) are popular both as foods and traditional remedies. Despite their widespread use, little is known about the combined effects and molecular mechanisms of ethanol extracts from passion fruit seeds and water extracts from roselle, especially when administered with standard medications. This study therefore aims to evaluate the efficacy and underlying molecular pathways of these extracts, alone and in combination with metformin and atorvastatin, in an *in vitro* model of hepatosteatosis. HepG2 cells were exposed to oleic acid and high-glucose culture media to induce fatty liver conditions. Mechanistically, the extracts reduced intracellular reactive oxygen species (ROS) and apoptosis-related cell death. They also decreased triglyceride accumulation by downregulating *CD36* gene expression, thereby limiting fatty acid uptake. Additionally, the extracts suppressed acetyl-CoA carboxylase (ACC) expression and promoted beta-oxidation via activation of carnitine palmitoyl transferase 1A (CPT1A). Synergistic activation of peroxisome proliferator-activated receptor gamma coactivator-1α (PGC1α) and nuclear respiratory factor 1 (NRF-1) further contributed to reduced lipid accumulation. Furthermore, the extracts inhibited the Akt/mTOR signaling pathways and suppressed nuclear factor-kappa B (NF-κB) activation, leading to decreased production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, and IL-1β. These findings suggest that passion fruit seed and roselle extracts, alone or in combination with standard drugs, may serve as therapeutic agents against fatty liver disease by targeting oxidative stress, inflammation, and lipid metabolism.

Keywords: *Passiflora edulis*, *Hibiscus sabdariffa*, non-alcoholic fatty liver disease, free radical, apoptosis

Sangyod Rice and Rice Bran Extracts Attenuate Inflammation in Skin Keratinocytes

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Abstract

Skin inflammation arises from exposure to allergens, irritants, or pathogens, with epidermal keratinocytes playing a pivotal role by releasing pro-inflammatory cytokines and chemokines. Sangyod rice (*Oryza sativa* L.), a red-pigmented variety cultivated in Phatthalung province, contains bioactive compounds in its bran-traditionally discarded as animal feed-that exhibit anti-inflammatory, antioxidant, and antimicrobial properties. This study evaluated the effects of Sangyod rice extract (SRE) and its bran hydrolysate (SRB-H) on lipopolysaccharide (LPS)-induced inflammation in human keratinocytes (HaCaT). Cytotoxicity assays confirmed both SRE and SRB-H were non-toxic at concentrations up to 1000 µg/mL. At concentrations of 50–100 µg/mL, SRE and SRB-H attenuated LPS-induced inflammation by reducing reactive oxygen species (ROS) and nitric oxide (NO) production. SRE and SRB-H reduced apoptotic cell death by downregulating caspase-3 expression. ELISA analysis revealed significant reductions in TNF-α and IL-6 levels, demonstrating anti-cytokine activity. Mechanistically, SRE and SRB-H inhibited LPS-induced activation of the Akt and p38 MAPK pathways, key regulators of inflammatory signaling. Immunofluorescence and western blot analyses further demonstrated suppressed nuclear factor-kappa B (NF-κB) nuclear translocation and reduced expression of toll-like receptor 4 (TLR4) and inducible nitric oxide synthase (iNOS), accompanied by decreased production of pro-inflammatory mediators. The findings underscore SRE and SRB-H as potent inhibitors of keratinocyte-mediated inflammation, primarily through modulation of the Akt/p38 MAPK/NF-κB axis. By targeting multiple inflammatory pathways, Sangyod rice derivatives offer promising therapeutic potential for skin conditions such as atopic dermatitis, where keratinocyte dysregulation contributes to pathology. Further research is warranted to validate these effects in clinical settings and to optimize formulations for topical or systemic application.

Keywords: Sangyod rice, Sangyod rice bran, lipopolysaccharide, human epidermal keratinocytes, HaCaT

Validation of Mouse Xenograft Model for Non-Small Cell Lung Cancer Using Combination of Conventional Tumor Volume Measurement and Bioluminescence *in-vivo* Imaging

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Abstract

Lung cancer is a major public health concern in Thailand and worldwide, with the highest prevalence and mortality among the ten most common cancers. Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer cases. In drug discovery and development, preclinical *in-vivo* studies, such as the mouse xenograft model, are essential for evaluating the efficacy of candidate drugs before clinical trials. Tumor volume measurement by caliper is a conventional method, while bioluminescence *in-vivo* imaging, which detect luciferase activity in tumor cell by converting D-luciferin to Oxyluciferin and light detected by special camera is beneficial for monitoring tumor growth in cancer study, offers real-time tumor monitoring. This study aims to determine the optimal NSCLC cell number for a mouse xenograft model by using both measurement techniques. A luciferase-expressing A549 cell line (A549-luc) was injected subcutaneously into the right flank of four BALC/cAJcl-nu (nude) mice at two doses: $2x10^6$ cells and $5x10^6$ cells. Tumor growth was monitored biweekly using caliper to measure tumor volume (TV), calculated as 0.5 x length x width^2. Additionally, biweekly imaging was performed using the IVIS Spectrum *In-Vivo* Imaging System. Mice were injected intraperitoneally with 150 mg/kg luciferin, and imaging was conducted 10 minutes later under isoflurane anesthesia. Image was captured by IVIS CCD camera and analyzed for bioluminescence intensity, expressed as photons per second (p/s), using the living image software. The results showed that tumor volume reached 50 - 100 mm^3 within four weeks in $5x10^6$ cells group and within five to six weeks in the $2x10^6$ cells group. Moreover, the $5x10^6$ cells group exhibited a more consistent tumor growth rate, both in volume and bioluminescence intensity, compared to the $2x10^6$ cells group. In conclusion, $5x10^6$ A549-luc cells provide an optimal tumor growth rate, making this dosage suitable for xenograft models in lung cancer research.

Keywords: Non-small cell lung cancer (NSCLC), xenograft, nude mice, bioluminescence imaging, tumor volume

A08

Pharmacokinetic Profiling of Plant-Derived M2 Monoclonal Antibody Against Botulinum Toxin Type B in a Mouse Model

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Abstract

Botulism is a potentially fatal illness caused by the neurotoxins produced by *Clostridium botulinum*, which was responsible for a significant outbreak in 2006 and remains a public health concern in Thailand. The botulinum toxin affects the nervous system, leading to difficulty breathing, muscle paralysis, and, in severe cases, death. Among the various toxin serotypes, botulinum neurotoxin types A and B are the primary causes of foodborne botulism in humans, with symptoms that may persist for weeks or months. The standard treatment, botulinum antitoxin, is crucial for reducing mortality; however, its availability is limited in many countries, including Thailand. Plant-produced monoclonal antibodies (mAbs) have emerged as a promising therapeutic approach. The M2 mAb, produced in plants, has demonstrated high affinity for binding to botulinum toxin type B. This study aimed to determine the pharmacokinetic profile of plant-produced M2 mAbs following intravascular administration in a mouse model. Male ICR mice (n=12/group) were administered M2 mAbs at a dose of 100 µg/mouse. Blood samples were collected from subgroups of four mice at 12 time points, and serum M2 mAb concentrations were analyzed using ELISA technique. Pharmacokinetic analysis, performed using PKSolver program, showed rapid absorption (t_{max} = 0.17 h) and a prolonged elimination half-life ($t_{1/2}$ = 86.36 h). The area under the concentration-time curve (AUC) was 1183.38 µg*h/mL, indicating sustained systemic exposure. These results suggest that M2 mAbs are a long-acting monoclonal antibody with the potential for an infrequent dosing regimen while maintaining therapeutic efficacy. Further studies will focus on evaluating its neutralization potency and therapeutic efficacy. These pharmacokinetic findings support the continued development and clinical investigation of plant-produced anti-botulinum toxin type B mAbs.

Keywords: Botulinum neurotoxin type B, M2 monoclonal antibody, mouse, pharmacokinetics, plant-derived

A09

Preliminary Study of the Anti-Ulcerative Colitis Effects of *Pluchea indica* Leaf Extract in a Rat Model

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Abstract

Ulcerative colitis (UC) is a chronic inflammatory condition that affects the colon. Although current treatments primarily focus on managing symptoms and controlling inflammation, there is no definitive cure. *Pluchea indica*, a plant containing a variety of bioactive compounds, has shown anti-inflammatory properties. However, specific studies examining its potential for treating UC are lacking. This study aims to investigate the effects of *P. indica* leaf extract (PIE) on its anti-ulcerative colitis activity in a rat model of dextran sulfate sodium (DSS)-induced colonic inflammation. Wistar rats were assigned to four groups: control, DSS, treatment (PIE), and sulfasalazine (SSZ) as anti-inflammatory drug. Prior to colitis induction, rats in the control and DSS groups received distilled water (DW), while those in the PIE and SSZ groups were orally administered PIE (150 mg/kg) and SSZ (30 mg/kg), respectively, for 14 consecutive days. On day 7 of treatment, colitis was induced in the DSS, PIE, and SSZ groups by providing 3% DSS in drinking water for 7 days. The severity of UC was assessed daily based on body weight loss, fecal consistency and bleeding. The DSS group exhibited significant body weight loss, altered fecal consistency, and increased fecal bleeding ($p < 0.05$) compared to control group, all these changes indicated the severity of colitis. Histological analysis of the colon using H&E staining revealed tissue injuries in the DSS group, characterized by epithelial damage and crypt abnormalities. In contrast, the PIE-treated group showed significant improvements in body weight loss, fecal consistency, and bleeding ($p < 0.05$) compared to DSS group. Furthermore, both the PIE- and SSZ-treated groups exhibited a marked reduction in histopathological alterations. These findings suggest that PIE has notable anti-ulcerative colitis effects and support its development as a natural alternative medicine for the prevention of colonic inflammation, particularly UC.

Keywords: *Pluchea indica*, ulcerative colitis, colonic inflammation, dextran sulfate sodium

Effect of Inhaled β-Caryophyllene on Nicotine-induced Vascular Degeneration

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Abstract

β-caryophyllene (BCP) is a volatile bicyclic sesquiterpenoid commonly found in various spices. It has been reported to act as an agonist of several receptors, including the cannabinoid type 2 (CB2) receptor. Orally administered BCP has been reported to have multiple physiological activities such as anti-inflammation, anti-oxidation, and analgesic effects. However, the effect of inhaled volatile BCP on biological activity remain largely unknown. We previously reported that inhaled BCP was transferred into the serum and organs of mice. In the liver of mice, dynamic changes of metabolites were observed, suggesting inhaled BCP can affect biological activities after incorporated into body. In this study, we evaluated the effect of inhaled BCP on nicotine-induced vascular degeneration. For the experiment, 4-week-old ddY mice were divided into 4 groups (Control group: no BCP inhalation + water, BCP group: 10 mL BCP inhalation + water, Nicotine group: no BCP inhalation + 0.5 mg/mL nicotine solution, Nicotine + BCP group: 10 mL BCP inhalation + 0.5 mg/mL nicotine solution). After 5 days of acclimatization, nicotine solution administration and BCP inhalation were initiated; BCP inhalation was performed three times a week for 60 minutes. Two weeks after the start of nicotine solution administration and BCP inhalation, aortae were collected for EVG staining, Immunohistochemical staining and vascular tensile rupture tests. In the nicotine group, destruction of elastic fiber and aortic stiffness of the aorta were induced with increased matrix metalloproteinase-2 (MMP-2), an enzyme that degrades elastic fibers. These were attenuated by BCP inhalation. BCP was detected in the aorta of animals which inhaled BCP. The rate of elastic fiber destruction of BCP in cultured *ex vivo* aortae were blocked by AM630, an inhibitor of the CB2 receptor. These results suggest that inhaled BCP can attenuate nicotine-induced aortic degeneration.

Keywords: β-caryophyllene, vascular degeneration, inhalation

Abdominal Aortic Hypoperfusion Can Induce Medial Arterial Calcification in Aorta

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Abstract

Medial arterial calcification is a chronic systemic vascular disorder. The hallmark of medial arterial calcification is ectopic deposition of calcium phosphate within the medial layer of the aorta. Medial arterial calcification causes arterial stiffness which leads to several cardiovascular diseases including heart failure, left ventricular hypertrophy, and diastolic dysfunction. The development of medial arterial calcification has been reported to be associated with aging, diabetes, and chronic kidney disease. However, the mechanisms underlying the development of medial calcification remain unclear. In this study, we found that aortic hypoperfusion can induce medial arterial calcification. Six-week-old male Sprague-Dawley rats were catheterized into the abdominal aorta and ligated the aorta on the catheter to induce aortic hypoperfusion. After the treatment, the rats were dissected at 6, 12, 24, and 48 hours, and the abdominal aorta was analyzed pathologically and observed using a scanning electron microscope (SEM/EDS). The area of calcium deposition significantly increased 24 and 48 hours after treatment compared to the control group. The rate of destruction of elastic fibers significantly increased after 6, 12, 24, and 48 hours of treatment compared with the control group. The area of positive areas for nucleus in the arterial media was significantly reduced after 6, 12, 24, and 48 hours of treatment compared with the control group. Calcium phosphate crystals were deposited within the medial layer of the aorta. These data suggest aortic hypoperfusion can induce medial arterial calcification.

Keywords: Aortic medial calcification, vascular degeneration, animal models

F01

Study of the Free Radical Scavenging Capacity of Standardized *Centella asiatica* (ECa 233) Extract and its Triterpenoids by Electron Paramagnetic Resonance Spectroscopy

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Abstract

Centella asiatica is an herbaceous plant that has been used traditional medicine in several cultures. Recently, its standardized extract named ECa 233 contains >80% w/w of its pentacyclic triterpenoids which have been reported to exhibit antioxidant activity; however, its mechanisms of action remain unclear. This study investigates the radical scavenging activity of ECa 233 and its pentacyclic triterpenes (asiatic acid, asiaticoside, madecassic acid, and madecassoside) against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals (·OH) using electron paramagnetic resonance (EPR) spectroscopy. For DPPH radical scavenging activity, ECa 233 exhibited a half-maximal inhibitory concentration (IC_{50}) of approximately 7.8 mg/ml accounting for Trolox equivalent antioxidant capacity of 4.2 nmol per ECa 233 at 1 mg. It also showed hydroxyl radical scavenging activity, with an IC_{50} of 3.8 mg/ml. These findings suggest that ECa 233 inhibits the Fenton reaction. In contrast, the pentacyclic triterpenes demonstrated no scavenging activity in either assay at their highest concentration.

Keywords: antioxidant, *Centella asiatica*, iron chelators, EPR spectroscopy

Introduction

Centella asiatica (L.) Urban (known as bua-bok in Thai) is a perennial herb widely distributed in tropical regions. Traditionally, its leaves have been used in medicine across various cultures, such as Chinese, French Pharmacopoeia, and Indian Ayurveda. Its therapeutic effects have been recommended for skin diseases, cognitive improvement.[1] Research has identified that the primary active compounds in the standardized extract of *C. asiatica*, known as ECa 233, include ≥ 80% w/w of pentacyclic triterpenoid glycosides, with a ratio of 1.5 ± 0.5:1 of madecassoside to asiaticoside.[2] Its bioactive compounds have demonstrated therapeutic potential against several diseases, including neurodegenerative, dermatological, and cardiovascular disorders. Therefore, their underlying mechanisms are proposed to be related to antioxidant and anti-inflammatory effects.[1] Additionally, studies have confirmed that ECa 233 is safe with minimal toxicity in both animal and human models.[3-4]

Previous research on ECa 233 in an animal model of β-thalassemia revealed a significant reduction in iron accumulation and oxidative stress (submitted manuscript). However, the precise mechanisms underlying its iron-chelating properties, as well as those of its key active compounds, remain unclear. While the antioxidant potential of *C. asiatica* extract, including ECa 233 and its main components, has been assessed in several studies, their specific radical scavenging properties have not yet been fully characterized.

Electron paramagnetic resonance (EPR) spectroscopy is a highly sensitive and precise technique for analyzing compounds with unpaired electron spins by detecting their distinct EPR spectra. This method has been widely applied in various fields, including drug discovery, particularly for evaluating antioxidant activity. Using the spin trapping method, EPR spectroscopy enables the quantification and characterization of radical scavenging activity in different compounds, making it a valuable tool for measuring interactions with various radical species.[5]

In this study, the free radical scavenging activity of ECa 233 and the primary bioactive compounds of *C. asiatica*, including asiatic acid, asiaticoside, madecassic acid, and madecassoside, were investigated against DPPH radicals and hydroxyl radicals using EPR spectroscopy. The findings from this research may provide further insights into the mechanisms of action of ECa 233 and its active compounds in mitigating oxidative stress-related diseases, and whether triterpenoids or other compounds contribute to the antioxidant activity in the extract.

Methods

ECa 233 and its pentacyclic triterpenoids

ECa 233 (Siam Herbal Innovation Co., Ltd., Thailand), asiatic acid (AA) and asiaticoside (AO) (Sigma, St. Louis, MO, USA), as well as madecassic acid (MA) and madecassoside (MO) (PhytoLab GmbH & Co. KG, Germany), were freshly prepared in 100% methanol.

DPPH scavenging assay

A stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma, St. Louis, MO, U.S.A.), was used to evaluate the radical scavenging activity of the test compound as a hydrogen donor. The 50 µl of 0.75 mM DPPH solution and 50 µl of the test compound, in total volume of 200 µl of methanolic solution, were combined in the reaction mixture. Test compounds were compared with Trolox as the standard antioxidant. The final concentration of ECa 233 ranged from 2–10 mg/ml, and the concentrations of other test compounds ranged from 3.125–1000 µM. The mixtures were transferred to capillary tubes and analyzed using an EPR spectrometer (E500, Bruker, U.S.A.). The EPR measurement conditions were as follows: central field 337±5 mT, modulation frequency 100 kHz, modulation amplitude 1.5 G, microwave power 7 mW, gain 11.5 dB, scan time 60 s, and recorded at 0, 2, 5, and 10 minutes.[6]

Hydroxyl radical scavenging assay

The Fenton reaction was used to generate hydroxyl radicals from ferrous sulfate and hydrogen peroxide. 5,5-Dimethyl-L-pyrroline-N-oxide (DMPO, Sigma, St. Louis, MO, U.S.A.) was purified by activated carbon and used as the trapping agent. The reaction mixtures were prepared in the following sequence: 10 µl of 0.25 mM ferrous sulfate, 10 µl of deionized water, 20 µl of 1.12 M DMPO, 10 µl of the test compound, followed by 50 µl of 0.25 mM hydrogen peroxide. The final concentration of ECa 233 ranged from 0.625–10 mg/ml, and the concentrations of other test compounds ranged from 12.5–2000 µM. Deferiprone (Def; iron chelator) and salicylic acid (SA; antioxidant) were used as positive controls for inhibiting the

Fenton reaction. DMPO-OH adducts were recorded as the EPR spectra after adding hydrogen peroxide. The EPR measurement conditions were as follows: central field 336.7±5 mT, modulation frequency 100 kHz, modulation amplitude 1.5 G, microwave power 5 mW, gain 13 dB, scan time 40 s, and recorded at 0, 40, 80, and 120 seconds.[6]

Data analysis

The area under the peak of the EPR spectra in the DPPH and hydroxyl radical scavenging assays was calculated using EPRStudio software (BRUKER Xepr, Bruker, U.S.A.) through double integration. The scavenging activity of the test compounds was expressed as an inhibition percentage by comparing the area under the peak of the control reaction with or without the test compounds. The IC_{50} value was obtained by comparing the inhibition percentage against the antioxidant concentration.

Results

DPPH radical scavenging activity

Figure 1 illustrates the typical EPR spectra of DPPH radicals in methanol. The radical scavenging activity of the test compounds was determined by the reduction in the area under the peak. The spectra remained stable until the addition of test compounds, with ECa 233 and Trolox exhibiting a noticeable reduction in peak area. IC_{50} calculations revealed that 1 mg/ml of ECa 233 had an equivalent hydrogen-donating capacity to 4.24 nmol of Trolox. Therefore, the Trolox equivalent of other test compounds can be determined from their EPR spectra. Additionally, the DPPH scavenging activity and percentage inhibition of the test compounds are summarized in **Table 1**.

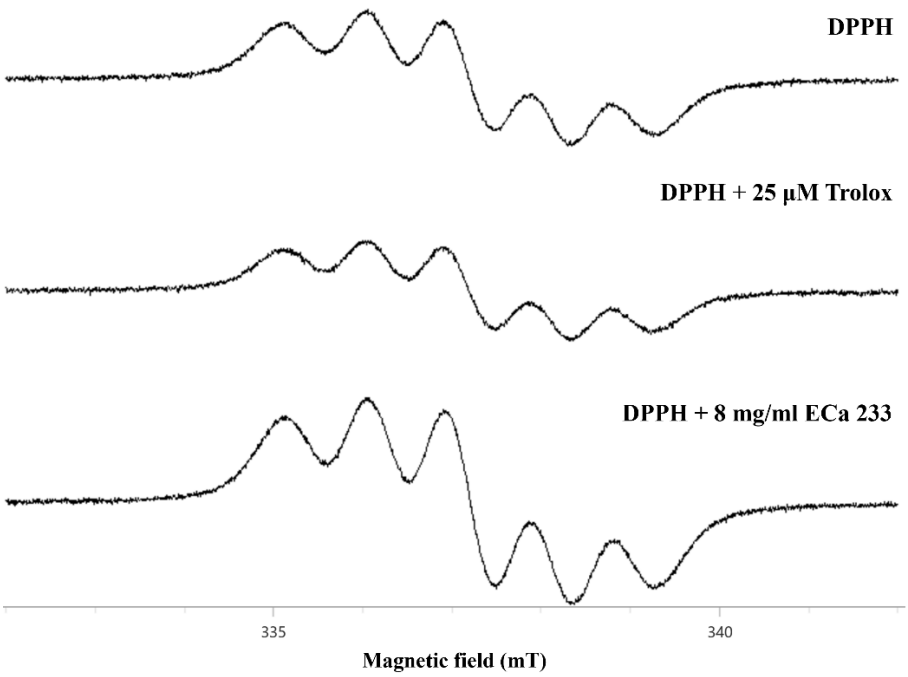

Figure 1. EPR spectra of DPPH radical, before and after addition of test compounds

Table 1. IC_{50}, percentage of inhibition of DPPH, and trolox equivalent capacity by ECa 233 and its pentacyclic triterpenoids

Test compound	DPPH		
	IC_{50}	% maximum Inhibition	Trolox equivalent capacity
Trolox	33.3 ± 0.9 µM	68.6 ± 1.6	-
ECa 233	7.9 ± 0.5 mg/ml	60.4 ± 0.7	4.24 nmol/mg
Asiatic acid	-	7.1 ± 2.2	-
Asiaticoside	-	4.9 ± 2.1	-
Madecassic acid	-	4.8 ± 0.2	-
Madecassoside	-	11.4 ± 0.4	-

DPPH radical scavenging activity of test compounds. Data represents the % maximum inhibition of the DPPH radical at 10 minutes for Trolox (50 µM), ECa 233 (10 mg/ml), and other test compounds (1000 µM). Values are expressed as mean ± S.D. from three independent experiments.

The DPPH radical scavenging kinetics of the test compounds were assessed to gain insight into their mechanisms of action (**Figure 2**). The scavenging activity of a mixture of MO and AO, at a ratio of 1.5 ± 0.5:1, was comparable to that of 1.2 mg/ml ECa 233 at similar concentrations. Overall, all test compounds followed a similar trend in DPPH radical scavenging activity. ECa 233 demonstrated a more pronounced reduction in the area under the peak compared to triterpenoids. However, despite reaching their maximum solubility in methanol, triterpenes exhibited lower hydrogen-donating activity than Trolox at its lowest concentration.

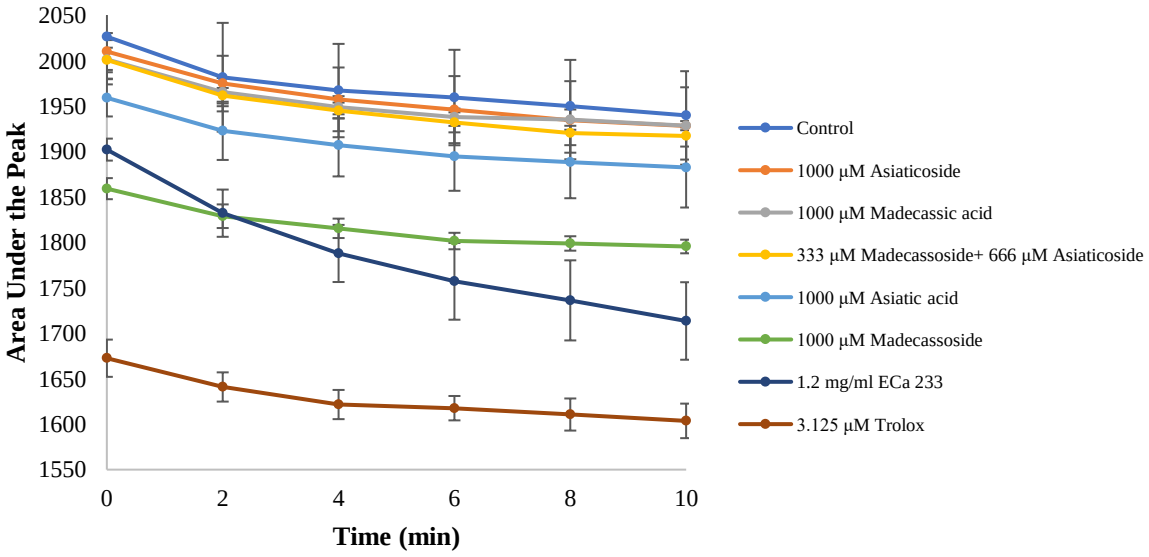

Figure 2. Kinetic curves of DPPH radical scavenging activity for each test compound at 0, 2, 4, 6, 8, and 10 minutes. Values are expressed as mean ± S.D. from three independent experiments.

Hydroxyl radical scavenging activity

The EPR spectra of hydroxyl radicals, generated by the Fenton reaction and detected using the spin-trapping technique with DMPO, exhibited a characteristic 1:2:2:1 pattern. Additional minor peaks were attributed to carbon-centered radicals due to the presence of methanol (**Figure 3**). The radical scavenging activity of the test compounds was evaluated

based on the reduction in the area under the peak. In the presence of deferiprone and ECa 233, a reduction in the area under the peak was observed immediately after the addition of the test compounds. In contrast, SA gradually inhibited hydroxyl radical formation, reaching maximum inhibition at 40 seconds post-addition. These results suggest that ECa 233 inhibits the Fenton reaction through the same mode of action as deferiprone, namely iron chelating activity.

IC_{50} calculations indicated that 1 mg/ml of ECa 233 exhibited a hydroxyl radical scavenging capacity equivalent to 10 nmol of SA and an iron chelation capacity equivalent to 21 nmol of deferiprone. Based on these findings, the EPR spectra of other test compounds can be used to determine their hydroxyl radical scavenging activity, deferiprone equivalent, and percentage inhibition, as summarized in **Table 2**. The other triterpenes exhibited no scavenging activity, as evidenced by the absence of any reduction in the area under the peak (data not shown).

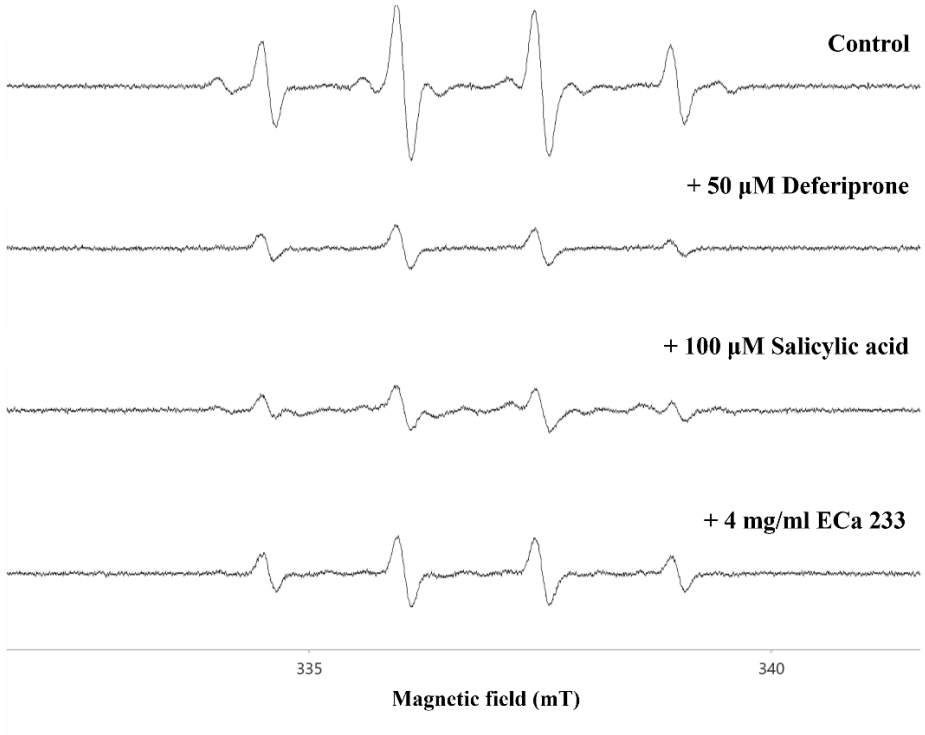

Figure 3. EPR spectra of hydroxyl radical, before and after addition of test compounds

Table 2. IC_{50}, Percentage of Inhibition of hydroxyl radical, and scavenging and iron chelation activity by ECa 233 and its derivatives

Test compound	Hydroxyl radical			
	IC_{50}	% Maximum inhibition	Deferiprone equivalent capacity	Scavenging capacity
Deferiprone	38 ± 3.9 µM	71.9 ± 1.0	-	-
Salicylic acid	-	62.4 ± 2.0	-	-
ECa 233	3.8 ± 0.2 mg/ml	65.8 ± 1.8	10 nmol/mg	21 nmol/mg
Asiatic acid	-	Nd	-	-
Asiaticoside	-	Nd	-	-
Madecassic acid	-	Nd	-	-
Madecassoside	-	Nd	-	-

Hydroxyl radical scavenging activity of test compounds. Data represents the % maximum inhibition of the hydroxyl radical at 40 seconds for SA (50 μM), at 0 seconds for ECa 233 (10 mg/ml), and for other test compounds (100-200 μM). Nd=Not detectable. Values are expressed as mean ± S.D. from three independent experiments.

Discussion

A previous study by Tamatam and Naika[7] on the antioxidative activity of *C. asiatica* in methanol solution demonstrated a concentration-dependent DPPH radical scavenging effect, reaching up to 2 μg/ml. In comparison, our study suggests that standardized extracts may exhibit higher radical scavenging activity than crude extracts due to the controlled presence and quality of bioactive compounds. Notably, this is the first EPR spectroscopy study on the standardized extract of *C. asiatica* (ECa 233), providing evidence that ECa 233 can scavenge reactive oxygen species through its hydrogen-donating properties and inhibition of the Fenton reaction.

Antioxidant and free radical studies are complex due to the unique characteristics of each reactive oxygen species, including their reactivity at low concentrations and extremely short half-lives. Conventional antioxidant assays may not always provide clear or comprehensive results, as they are often limited to specific experimental models. The development of EPR spectroscopy has expanded the ability to study a wide range of free radical species in different assay systems. Combined with spin-trapping techniques, which extend the half-life of radicals, EPR spectroscopy offers a highly sensitive method for characterizing radicals. Unlike traditional spectrophotometry, it is not affected by sample color interference, making it a powerful tool for antioxidant research.[5]

Previous *in vitro* and *in vivo* studies on metabolic syndrome and neurological diseases have shown that the pentacyclic triterpenoids in *C. asiatica* exhibit enzymatic antioxidant and anti-inflammatory activities through the upregulation of Nrf-2 and the reduction of inflammatory cytokines.[8] Our findings support these results, suggesting that ECa 233 and its triterpenes may help mitigate oxidative stress and inflammation through multiple pathways.

Additionally, our previous study in an animal model of β-thalassemia demonstrated a significant reduction in iron accumulation and oxidative stress following ECa 233 administration. The current study further clarifies the mechanism of ECa 233, highlighting its ability to inhibit the Fenton reaction. However, further research on the iron-chelating activity and effects on lipid radicals of ECa 233 and its triterpenes is needed to fully elucidate their mechanism of action.

Although ECa 233 demonstrated potent radical scavenging activity, its triterpenes did not exhibit significant radical inhibition, despite being major constituents. This suggests that the remaining 20% of ECa 233 may contain compounds with strong scavenging activity, warranting further investigation.

Conclusion

Our findings provide a qualitative analysis of the free radical scavenging activity of ECa 233 and its triterpenes. Although this study did not confirm significant radical scavenging activity of the triterpenes, their antioxidant properties may act through multiple pathways to combat oxidative stress-related diseases, such as metabolic syndrome, neurological disorders, and β-thalassemia.

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Effect of Various Concentrations of Ethanol on Antioxidant Activity of *Tiliacora triandra* Extract

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Abstract

Tiliacora triandra (Yanang) is a popular medicinal plant in Thailand and the Southeast Asia due to its numerous therapeutic properties, such as antipyretic, detoxification, and promoting overall health. Studies have shown that *T. triandra* contains various bioactive compounds, including phenolic compounds and flavonoids, which are antioxidants that play a crucial role in preventing cellular damage caused by free radicals. These compounds also have the potential to reduce the risk of diseases such as cardiovascular diseases, cancer, and other chronic conditions. This study aimed to investigate and compare phenolic compounds, flavonoids, and antioxidant activity levels in *T. triandra* roots and leaves. The results showed that roots of *T. triandra* had higher phenolic content, total flavonoid content, and antioxidant activity than its leaves. From the experiments, it was found that 80% ethanol (EtOH) was the most effective solvent in root extracts, yielding the highest phenolic content (33.2 ± 1.5 mg/g gallic acid equivalent) and the highest flavonoid content (1.26 ± 0.03 mg/g quercetin equivalent). Thus, it can be concluded that 80% EtOH extract of *T. triandra* root exhibited the strongest antioxidant activity (72.68 ± 0.57 %) by using DPPH assay. The findings from this study will contribute to a better understanding of *T. triandra* and promote its future use in healthcare and the herbal industry.

Keywords: *Tiliacora triandra*, Phenolic compounds, Flavonoids, Antioxidant activity

Introduction

Antioxidants, in chemical terms, are compounds that can prevent or slow down the process of oxidation. Oxidation occurs in various forms, such as the process that causes iron to rust, makes apples turn brown, or causes vegetable oil to become rancid. Oxidation can also occur in the human body, such as the breakdown of proteins and fats from the food we consume, exposure to air pollution, breathing, cigarette smoke, and UV radiation, all of which generate free radicals that can damage the body.[1] No single compound can completely prevent oxidation. Different mechanisms may require different antioxidants to halt the oxidation process. Antioxidants can help reduce the risk of several diseases, especially chronic conditions like cancer, diabetes, cardiovascular diseases, and Alzheimer's disease. They also help slow down the processes that lead to aging. Normally, the body can remove free radicals before they cause harm, but if free radicals are produced too quickly or in excess, beyond

what the body can neutralize, it can negatively affect health. Free radicals are commonly found in nature, such as in phenolic compounds, nitrogen compounds, flavonoids, and carotenoids. Phenolic compounds are substances found in many plants, such as pepper, chili, ginger, garlic, shallots, onions, legumes, seeds, grains, rice, and sesame.[2] These phenolic compounds have antioxidant properties, are water-soluble, and help protect against diseases such as coronary heart disease and cancer. They work by neutralizing free radicals and metal ions, which can accelerate the oxidation of fats and other molecules.

T. triandra, commonly known as Yanang, is a woody vine in the Menispermaceae family. Its round, flexible stems are green when young and turn gray as they mature. It is used to twine around other plants. The leaves are simple, and the flowers are arranged in branched panicles. The fruit is glossy green when immature and turns reddish-orange when ripe. The seed is hard, with a single seed that can be used for planting. The root of *T. triandra* has an underground tuber and is large in size. Its taste is bitter and slightly astringent, and it is used to relieve fever and treat various types of fevers, including intermittent fever, febrile diseases, red fever, malaria, and chickenpox. It is also used to detoxify the body, alleviate hangovers, improve heart function, and balance the body's elements.[3] Additionally, *T. triandra* root helps treat heart diseases, expel toxins, and relieve wind-related ailments. Although pharmacological investigations of this plant have been previously reported, data pertaining to its extraction using solvents of varying concentrations remain incomplete. Therefore, this study aimed to compare the amounts of phenolic compounds, flavonoids, and antioxidant activity in the root of *T. triandra*. The findings will help enhance the value of the plant and lead to commercial production, facilitating further research and development in the field.

Methods

Chemicals and reagents

Reference standards, gallic acid, and quercetin were obtained from Biopurify (Chengdu, China). 1,1-diphenyl-2-picrylhy-drazyl (DPPH) was obtained from Sigma (MO, USA). Folin- Ciocalteu phenol reagent and Griess reagent were purchased from Loba Chemie, Mumbai, India.

Plant extract preparation

Different parts of *T. triandra*, including leaves and roots, were collected at Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, Thailand, in January 2025. All samples were dried at 50°C for 48 h and ground into powder using a mortar and pestle. A sample powder (100 mg) of each part was extracted using distilled water and various concentration of EtOH (40% and 80%) in a sonic bath. The sample was centrifuged at 10,000 rpm in microcentrifuge, and the supernatant was collected. The extraction process was repeated four times. The extracts were evaporated and re-dissolved in 1 mL of various concentrations of EtOH, which were used in subsequent experiments. All extractions were performed in triplicate.

Quantification of total phenolic and flavonoid contents

The aluminium chloride colorimetric assay used by Amorim et al.[4] was employed to determine the total flavonoid content with slight modification. Standard solutions of quercetin (0.19–100 μg/mL) were prepared in methanol. The absorbance of the reaction mixture was measured at 405 nm using a microplate spectrophotometer. The total flavonoid content was estimated by comparing the absorbance value to the quercetin standard curve and expressed as milligrams of quercetin equivalent per gram of sample dry weight (mg QE/g DW). All analyses were performed in triplicate. The regression equation for the quercetin

standard curve was y=0.0105x+0.0646 and R^2=0.997.

The total phenolic content of *T. triandra* extracts was determined using the Folin-Ciocalteu colorimetric method described by Everette et al.[5] with slight modification. The *T. triandra* extracts or methanol (blank) were separately mixed with the Folin-Ciocalteu re-agent (1:5, v/v) in 96-well plates, shaken for 1 min, and kept in the dark at 25°C for 6 min. The mixture was added to 7% Na2CO3, incubated in the dark at 25°C for 30 min, and measured at 760 nm using a microplate reader. The total phenolic content was estimated by comparing the absorbance value to the gallic acid standard curve and expressed as milligrams of gallic acid equivalent per gram of sample dry weight (mg GAE/g DW). All analyses were performed in triplicate. The regression equation for gallic acid standard curve was y = 0.0041x + 0.1476 and R^2 = 0.9974.

DPPH radical scavenging capacity and FRAP assay

DPPH radical changes color from violet to shades of yellow after being quenched by an antioxidant. The antioxidant ability of AE extracts was determined by the DPPH radical scavenging assay. Briefly, a working DPPH (0.2 mM) solution was prepared in methanol. The DPPH solution was mixed with *T. triandra* extracts at different concentrations (1:1) or with methanol as control. The reaction mixtures were incubated at 25°C for 30 min in the dark. The mixture of each extract with methanol was represented as blank. The free radical scavenging capacity was determined by measuring the absorbance at 490 nm. Ascorbic acid was used as an antioxidant standard.[6]

Statistical analysis

All experiments were performed in triplicate and the data are expressed as the mean ± standard deviation (SD).

Results

The total phenolic contents of *T. triandra* roots and leaves extracted with distilled water, 40% EtOH, and 80% EtOH was showed in **Table 1**. The results showed that *T. triandra* roots extract has rich phenolic contents when compared with the leaves. *T. triandra* roots extracted with 80% EtOH had the highest total phenolic contents (33.2 ± 1.50 mg/g gallic acid equivalent).

Table 1. Total phenolic contents of *T. triandra* roots and leaves extracted with various extraction solvents

Extraction solvent	Total phenolic content mg/g gallic acid equivalent	
	***T. triandra* roots**	***T. triandra* leaves**
Distilled water	13.6 ± 0.50	0.01 ± 0.02
40% EtOH	13.1 ± 0.06	0.06 ± 0.01
80% EtOH	33.2 ± 1.50	1.85 ± 0.01

In addition, the total flavonoid contents of *T. triandra* roots and leaves extracted with various extraction solvents were also investigated. The results showed that *T. triandra* leaves extracted with 80% EtOH had the highest total flavonoid contents 3.71 ± 0.05 mg/g quercetin equivalent, as shown in **Table 2**.

Table 2. Total flavonoid content of *T. triandra* roots and leaves extracted with various extraction solvent

Extraction solvent	Total flavonoid content mg/g quercetin equivalent	
	***T. triandra* roots**	***T. triandra* leaves**
Distilled water	0.23 ± 0.07	0.03 ± 0.01
40% EtOH	0.55 ± 0.01	0.11 ± 0.01
80% EtOH	1.26 ± 0.03	3.71 ± 0.05

DPPH assay was used to investigate the antioxidant activities of *T. triandra*. In this study, the concentration of ascorbic acid standard was 0.1 mg/ml, while the roots extract and leaves extract concentrations in the DPPH assay were 0.5 mg/ml. The antioxidant activities of *T. triandra* were showed in **Table 3**.

Table 3. The percentage radical scavenging activity of *T. triandra* roots and leaves extract with various extraction solvent compared with ascorbic acid

Extraction solvent	DPPH scavenging activity (%)	
	***T. triandra* roots**	***T. triandra* leaves**
Distilled water	72.55 ± 0.74	71.52 ± 0.67
40% EtOH	72.31 ± 1.12	57.4 ± 0.67
80% EtOH	72.68 ± 0.57	70.85 ± 0.45

Discussion

The study found that 80% ethanol (EtOH) is the most effective solvent for extracting bioactive compounds from *T. triandra* roots. Specifically, 80% EtOH was able to extract the highest amounts of phenolic compounds and flavonoids compared to other solvents. The phenolic content extracted was as high as 33.2 ± 1.5 mg/g gallic acid equivalent, and the flavonoid content was 1.26 ± 0.03 mg/g quercetin equivalent. These findings are consistent with previous research indicating that phenolic compounds and flavonoids are important substances with strong antioxidant activity, playing a significant role in preventing cell damage caused by oxidation.[7-8] Phenolic compounds and flavonoids are secondary metabolites found in plants, known for their various biological activities, including antioxidant, anti-inflammatory, and anticancer properties.[9] Their extraction efficiency depends significantly on the solvent used, as different solvents have varying polarities that influence the solubility of these compounds. Studies have shown that aqueous ethanol solutions, particularly 70-80% ethanol, can maximize the extraction of phenolic and flavonoid compounds due to the combined solubility effects of both water and ethanol.[10] Regarding antioxidant activity, measured using the DPPH assay, the crude extract from *T. triandra* roots using 80% EtOH showed the highest antioxidant activity at 72.68 ± 0.57%. This was similar to other solvents but significantly higher than that obtained from distilled water and 40% ethanol. The DPPH assay is a widely used method for evaluating the free radical scavenging ability of natural compounds, and results from this study suggest that the phenolic and flavonoid content in *T. triandra* extracts directly contribute to their antioxidant potential.[11] The reason 80% EtOH yielded the best results may be due to the optimal proportion of water and ethanol, which effectively extracts both phenolic compounds and flavonoids, as they dissolve well in both water and alcohol. Other studies have also found that mixed solvents enhance the extraction

of bioactive compounds by improving solvent penetration into plant cells and increasing the diffusion rate of solutes.[12] This experiment highlights the significant impact of solvent selection on the efficiency of bioactive compound extraction and antioxidant activity. Specifically, mixed solvents like 80% EtOH can extract a wider range of compounds and are more effective than single solvents. Therefore, it can be concluded that 80% ethanol is the most suitable solvent for extracting *T. triandra* roots and leaves for various applications, such as producing extracts with high antioxidant activity for use in the food and pharmaceutical industries.[13]

Conclusion

The phenolic and flavonoid content in *T. triandra* root was higher than leaves. Therefore, the root is a more attractive resource than the leaves. 80% EtOH is the most suitable solvent for the extraction of *T. triandra* active compounds, especially phenolic and flavonoid, in both roots and leaves groups when compared with distilled water and 40% EtOH. The *T. triandra* leaves also had antioxidant property. However, the total phenolic and flavonoid contents of leaves are much lower than those of roots. To extend the current knowledge, future research should focus more on identifying and understanding other active compounds found in *T. triandra*. In addition, improving the extraction process should be explored further to increase both the number of useful compounds obtained and the potential health benefits of the extracts.

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F03

Strengthening CIK Cells via Dendritic Cells to Mitigate CIK Cell Exhaustion and Cellular Senescence Induced by Cholangiocarcinoma

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Abstract

Cytokine-induced killer (CIK) cells show promise in targeting cholangiocarcinoma (CCA), but their efficacy is limited by exhaustion and senescence. This study investigated whether co-culturing CIK cells with HubCCA1 cells induced these dysfunctions and explored counteractive strategies using mature dendritic cells (mDCs) and sunitinib-primed mDCs. Peripheral blood mononuclear cells (PBMCs) were isolated and used to generate CIK cells. $CD3^+CD56^+$ subset was isolated from CIK cells. CIK cells were pre-treated with mDCs or sunitinib-primed mDCs. Following pre-incubation, the CIK cells were exposed to cancer cells for 72 h. After exposure, phenotypic markers (CTLA-4, PD-1, TIM-3, KLRG1, CD28, CD57, perforin, and granzyme B) were analysed using flow cytometry. Flow cytometry analysis revealed that exposure to HubCCA1 cells reduced CIK cell viability and increased markers of senescence and exhaustion. However, pre-treatment with mDCs or sunitinib-primed mDCs mitigated these effects, preserving anti-tumor cytotoxicity of CIK cells. These findings suggested that mDC-based strategies may enhance the therapeutic efficacy of CIK cells in cancer treatment.

Keywords: Exhaustion, senescence, dendritic cells, sunitinib, CIK

List of abbreviations

APC	Allophycocyanin
APC-Cy7	Allophycocyanin-Cy7
CCA	Cholangiocarcinoma
CIK cells:	Cytokine-induced killer cells
E:T:	Effector to target ratio
FBS:	Fetal bovine serum
FITC:	Fluorescein isothiocyanate
FOXP3:	Forkhead box P3 (transcription factor)
GM-CSF:	granulocyte-macrophage colony-stimulating factor
HubCCA1:	Human cholangiocarcinoma cells
iDC	Immature Dendritic cells
IFN-Y:	Interferon-gamma

IL-2:	Interleukin 2
mDCs:	Mature Dendritic cells
mDCSu:	Mature Dendritic cells combine with Sunitinib
NK cells	Natural killer cells
OKT3:	Anti-CD3 monoclonal antibody
PBL:	Peripheral blood lymphocytes
PBMC:	Peripheral blood mononuclear cells
PBS:	Phosphate-buffered saline
PC5	Phycoerythrin-Cy5
PC5.5	Phycoerythrin-Cy5.5
PC7	Phycoerythrin-Cy7
PE:	Phycoerythrin
PerCP	Peridinin-Chlorophyll-Protein Complex
RPMI:	Roswell Park Memorial Institute (medium)
Th1 cells:	T helper 1 cells
TNF-α:	Tumor necrosis factor alpha

Introduction

Cholangiocarcinoma (CCA) is an aggressive malignancy of the biliary epithelium with high recurrent rate and limited response to conventional therapies.[1] One promising avenue of research in cancer therapy is cellular immunotherapy using cytokine-induced killer (CIK) cells. CIK cells have shown promise in targeting tumors like CCA. CIK cell, especially the most potent $CD3^+CD56^+$ subset, was especially effective due to its dual T cell and NK cell functionality, exerting strong anti-tumor activity through non-MHC-restricted[2] cytotoxicity, and IFN-γ secretion.[3]

However, the therapeutic efficacy of CIK cells in CCA has often been interrupted by immune dysfunction, particularly exhaustion and senescence. T cell exhaustion is a state of dysfunction arising from chronic antigen stimulation in the tumor microenvironment, leading to a progressive loss of T cell function.[4] Exhaustion has been characterized by the upregulation of inhibitory receptors such as PD-1, CTLA-4, and Tim-3, which impaired cytokine production and cytotoxic activity.[5] On the other hand, T cell senescence has been observed in the natural lifespan or aging process of the immune system. T cell senescence could be identified by the downregulation of costimulatory molecules (CD27 and CD28), and the upregulation of CD57 and KLRG-1.[6] These phenomena might contribute to the limited effectiveness of CIK cells in combating CCA. Given the significant challenges posed by exhaustion and senescence, there is a need to develop strategies to either prevent or reverse these dysfunctions in CIK cells. Dendritic cells (DCs) have been described as professional antigen-presenting cells that played a significant role in both immune tolerance and the response to cancer.[13] Enhancement of anti-tumor cytotoxic activity of CIK cells after exposure to DCs was due to the improved activation and expansion of tumor-specific T cells.[7] Sunitinib, a multi-targeted tyrosine kinase inhibitor, has shown promising results in the treatment of advanced cholangiocarcinoma. Its ability to simultaneously inhibit key pathways involved in tumor growth and angiogenesis makes it a rational therapeutic option for this aggressive cancer.[8-9]

We have reported sunitinib-primed mDCs enhanced the anti-tumor activity of CIK cells by promoting Th1 markers, leading to a stronger anti-tumor response.[10-11] This study investigated whether co-culturing CIK cells with HubCCA1 would induce exhaustion or senescence, particularly in the $CD3^+CD56^+$ subset. It also explored a potential strategy to

counter this dysfunction through the pre-incubation of CIK cells with either mDCs or sunitinib-primed mDCs. The changes in biomarkers associated with T cell exhaustion and senescence were monitored whether this treatment could restore the efficacy of CIK cell-based immunotherapy against CCA. The findings of this study would provide insight into the development of more effective therapeutic strategies.

Methods

Ethic statement

The studies have been approved by the Siriraj Institutional Review Board (SIRB) of the Faculty of Medicine, Siriraj Hospital, Mahidol University (COA no Si 213/2010).

Generation of CIK cells

PBMCs were isolated from healthy donor blood using Ficoll density gradient centrifugation. After washing, cells were counted, seeded at 2×10^6 cells/mL, and incubated for 2 h at 37°C, 5% CO_2. Non-adherent cells (peripheral blood lymphocytes, PBLs) were collected and cultured in RPMI-1640, 10% FBS, penicillin, streptomycin, 1000 IU/mL human recombinant IFN-γ (R&D Systems, Canada). PBLs were adjusted to 1×10^6 cells/mL and incubated for 24 h at 37°C, 5% CO_2. After incubation, 50 ng/mL anti-CD3 monoclonal antibody (OKT3) (BioLegend, USA) and 300 IU/mL IL-2 (Amoytop Biotech, China) were added. The culture was continued for 21 d to generate CIK cells, with 300 IU/mL IL-2 added every 2-3 d until harvesting.

Purification of $CD3^+CD56^+$ subset

After generating CIK cells for 2 weeks, $CD3^+$ cells were isolated using CD3 MicroBeads (Miltenyi Biotec, Germany). The $CD3^+$ cells were isolated for $CD3^+CD56^+$ subset using CD56 MicroBeads (Miltenyi Biotec, Germany).

Generation of mature dendritic cells

After PBMC preparation, the adherent monocytes were collected and incubated in RPMI-1640, 10% FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin. The monocytes were cultured at 37°C with 5% CO_2 for 2 weeks. They were induced into immature dendritic cells (iDCs) with 400 U/mL GM-CSF (Amoytop Biotech, China) and 500 IU/mL IL-4 (Amoytop Biotech, China) for 5-7 d. The maturation to mature dendritic cells (mDCs) was induced by 10 ng/mL TNF-α (Amoytop Biotech, China) for 24-48 h. For sunitinib-priming condition, 1 µM sunitinib (Sigma, St. Louis, MO) was added 1 d before maturation induction.

CIK cells pre-treated with mature dendritic cells (mDC) or combined with sunitinib exposure to HubCCA1 cells

In this experiment, CIK cells were first activated by co-culturing with mDCs or Sunitinib-primed mDCs at a 10:1 ratio for 5 d. After activation, the CIK cells were collected, centrifuged, and resuspended in fresh RPMI-1640. These pre-treated cells were then added to HubCCA1 at a 10:1 effector-to-target (E:T) ratio and incubated for 72 h. As a control, untreated CIK cells were also co-cultured with HubCCA1 under the same condition.

Cell viability using trypan blue exclusion

After co-culture, the cell suspension was gently removed, centrifuged, and resuspended. An aliquot (10 µL) was stained with trypan blue and counted using a hemocytometer to determine the number of viable CIK cells.

Flow cytometry analysis

After 72-h co-culture with HubCCA1, CIK cells were harvested and washed. Cells were stained for CD3, CD56, and exhaustion/senescence markers (e.g., CTLA-4, PD-1) (BioLegend, U.S.). Staining was performed at room temperature in the dark for 30 min. Cells were then fixed and permeabilized and incubated for 20 min in the dark. Intracellular staining for perforin (BioLegend) and granzyme B (R&D Systems) was performed for 1 h. Finally, cells were washed and analyzed using flow cytometry (CytoFLEX, Beckman Coulter).

FACS Analysis

Flow cytometry data were analyzed using FlowJo v10.7.1. Singlets were selected by gating FSC-A vs. FSC-H to exclude doublets, and debris was excluded based on FSC and SSC characteristics. The selected population was used for further analysis. Marker-positive populations were identified by comparison with unstained controls, and the percentage of positive cells was calculated for each marker, including CTLA-4, PD-1, Tim-3, KLRG1, CD28, CD57, perforin, and granzyme B.

Statistical Analysis

Data from experiments performed in triplicate were normalized by setting the 0-h value of each condition to 1. Fold-changes at subsequent time points were calculated relative to their respective baseline values. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test or an unpaired two-tailed t-test for pairwise comparisons. Graphs were generated using GraphPad Prism v8.3.0 (GraphPad Software, San Diego, USA). A p-value < 0.05 was considered statistically significant.

Results

Modulation of $CD3^+CD56^+$ population during CIK cell generation

From the analysis of alterations in cell proportions during CIK cell generation, three distinct data sets represent different cell subsets ($CD3^+CD56^+$, $CD3^-CD56^+$, and $CD3^+CD56^-$). The $CD3^+CD56^+$ subset gradually increased during CIK cell generation, peaking on day 20. The $CD3^+CD56^-$ subset rose during the first 3 d and gradually decreased, while the $CD3^-CD56^+$ subset was relatively stable (**Figure 1A**).

Cell viability

CIK cells initially exhibited ~95% viability before co-culture. At 24 h, viability slightly dropped to 86.7–90.1%, and at 48 h, it remained around 93–94.6%. A more significant decline was observed at 72 h (76.7–82.3%). Exposure to cancer cells resulted in a gradual decrease in viability over time, though not statistically significant, especially in CIK cells pre-treated with Sunitinib-primed mDCs (**Figure 1B**).

Phenotype analysis of $CD3^+CD56^+$ subset after exposure to HubCCA1

Purified $CD3^+CD56^+$ cells were co-cultured with HubCCA1 cells for 72 h and analyzed for CTLA-4, PD-1, TIM-3, KLRG1, CD28, CD57, perforin, and granzyme B (**Figure 2**). The analysis revealed a significant upregulation of CD57 ($p < 0.05$), granzyme B ($p < 0.05$), and a downregulation of CTLA-4 ($p < 0.05$), PD-1 ($p < 0.01$), Tim-3 ($p < 0.01$) and KLRG1 ($p < 0.001$) in $CD3^+CD56^+$ cells after co-culture, compared to untreated $CD3^+CD56^+$ cells at 72 h (**Figure 3**).

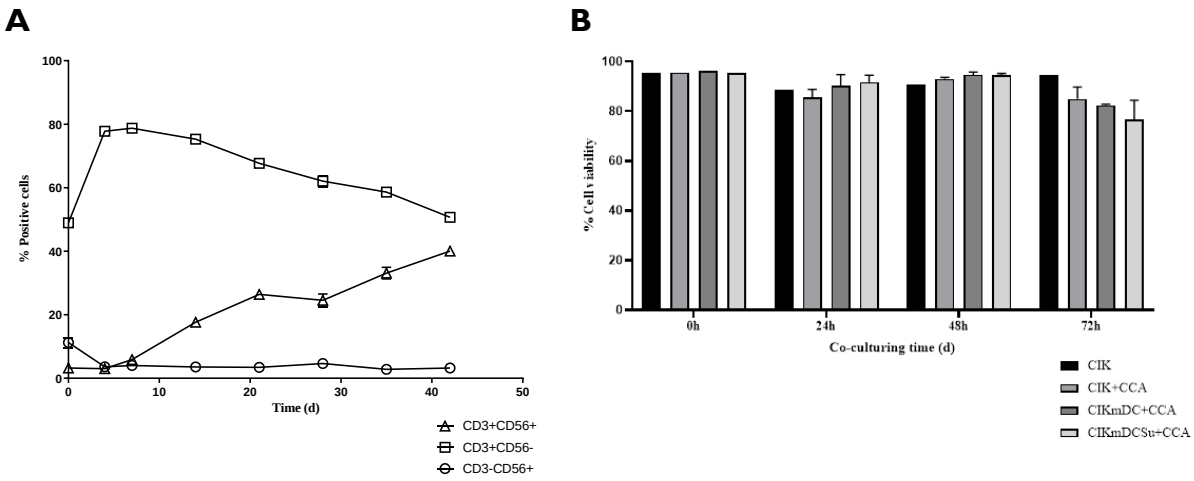


Figure 1. The FACS analysis for the alteration in the proportion of $CD3^+CD56^+$. The proportions of $CD3^+CD56^-$, $CD3^+CD56^+$, and $CD3^-CD56^+$ subsets were analyzed at different time points during CIK generation (A). The viability of CIK cells was assessed after being exposed to HubCCA1 cells for 0, 24, 48, and 72 h was monitored using a hemocytometer. Statistical analysis using two-way ANOVA followed by Tukey's test for multiple comparisons revealed no significant differences between conditions or time points (B).

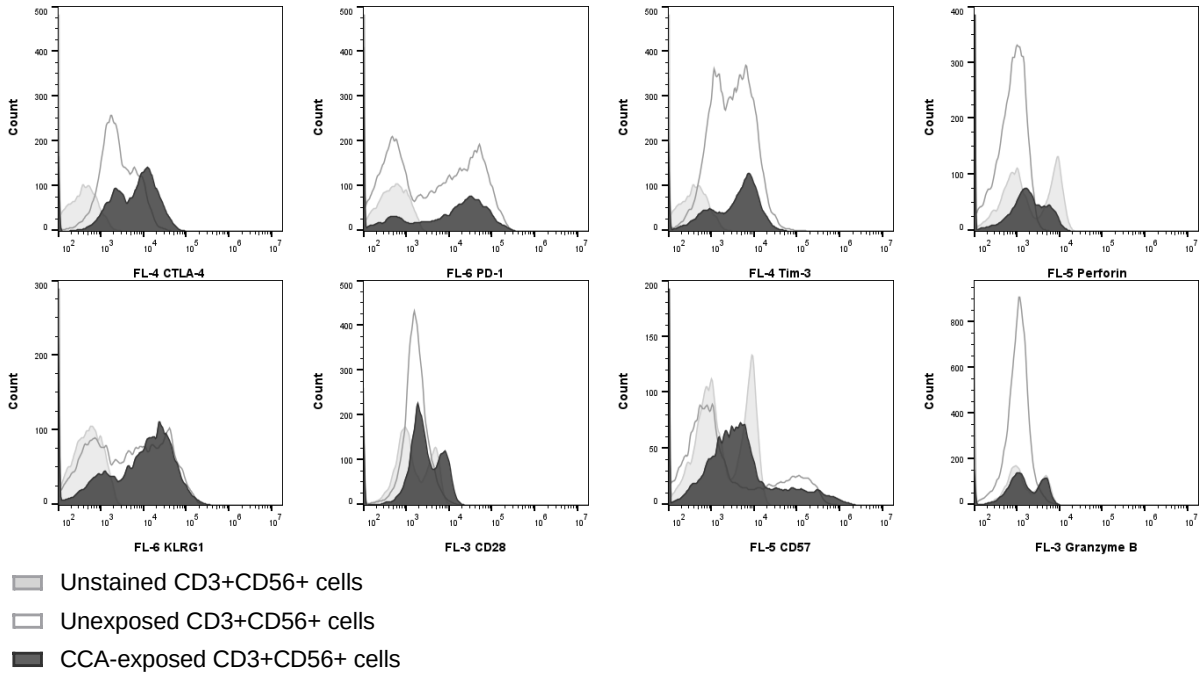


Figure 2. The histogram demonstrated phenotypic changes on $CD3^+CD56^+$ cells after 72 h exposure to cancer cells.

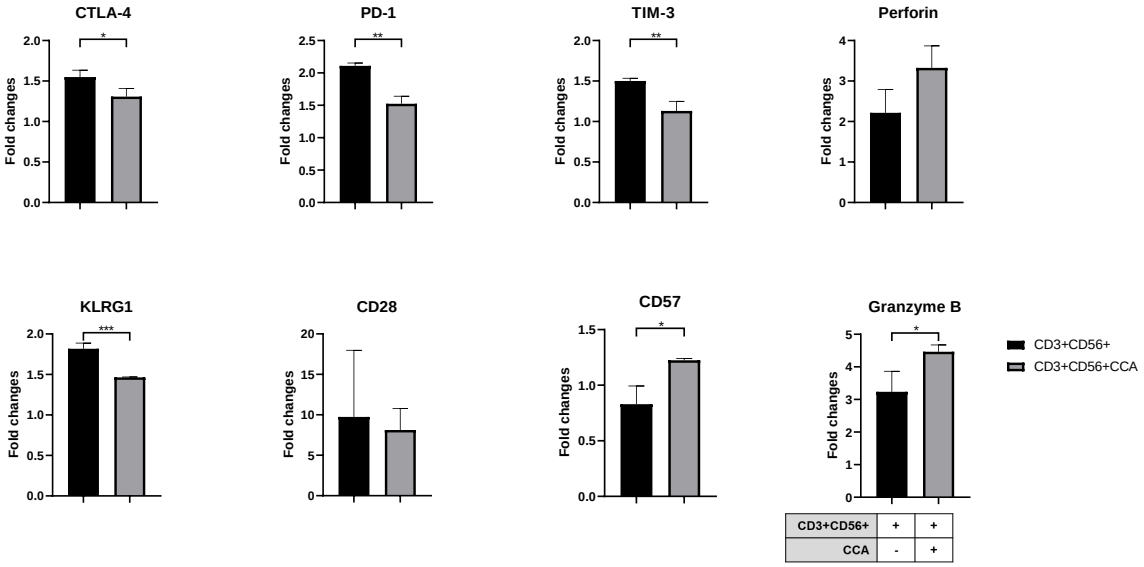


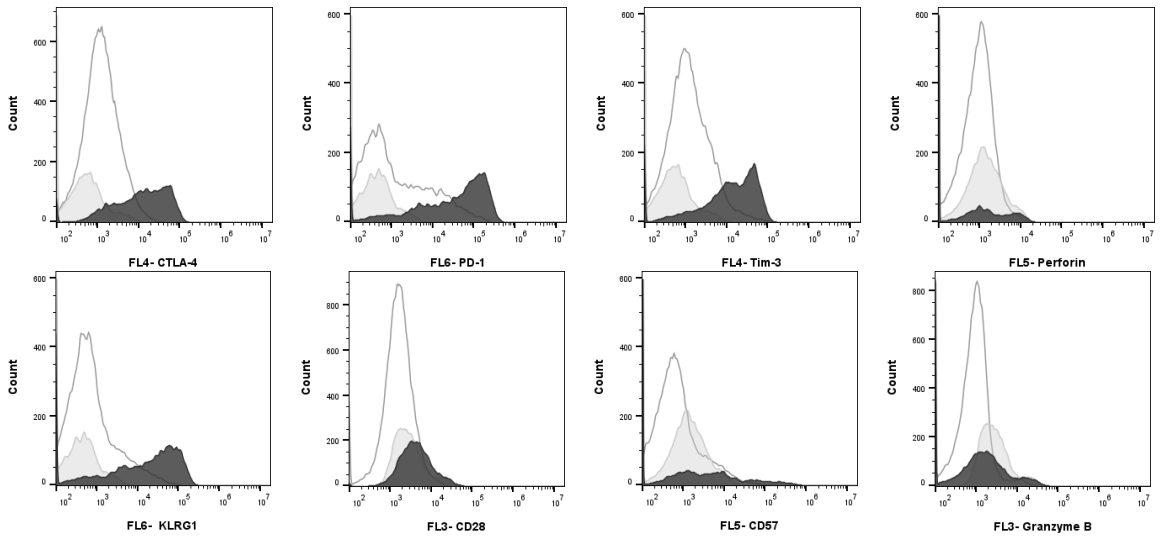
Figure 3. The phenotypic changes of $CD3^{+}CD56^{+}$ cells were assessed after 72 h exposure to HubCCA1. Normalized data were analyzed using an unpaired *t*-test with a 95% confidence interval. Significance levels were indicated as follows: * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$), and **** ($P < 0.0001$).

CIK pretreated with mDCs or sunitinib-primed mDCs partially prevented dysfunction from HubCCA1 exposure

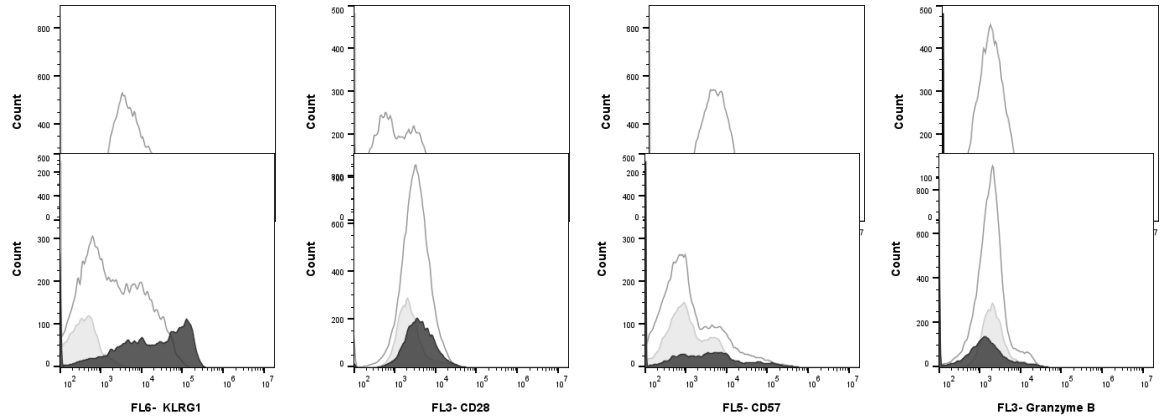
CIK cells were categorized under the following conditions: CIK, CIK+CCA, CIK+mDC+CCA, and CIK+mDCSU+CCA (**Figure 4**). The CIK+CCA group showed a significant increase in the levels of exhaustion markers CTLA-4 ($p < 0.05$), PD-1 ($p < 0.0001$), and Tim-3 ($p < 0.0001$); senescence markers KLRG1 ($p < 0.01$), CD28 ($p < 0.05$), and CD57 ($p < 0.05$); as well as perforin ($p < 0.01$) and granzyme B ($p < 0.0001$) (**Figure 5**), when compared to the CIK group.

Pretreatment of CIK cells with mDCs or sunitinib-primed mDCs (mDCSU) showed that both CIK+mDC+CCA and CIK+mDCSU+CCA significantly reduced the levels of CTLA-4, PD-1, Tim-3, KLRG1, CD28, perforin, and granzyme B, but not CD57 (**Figure 5**).

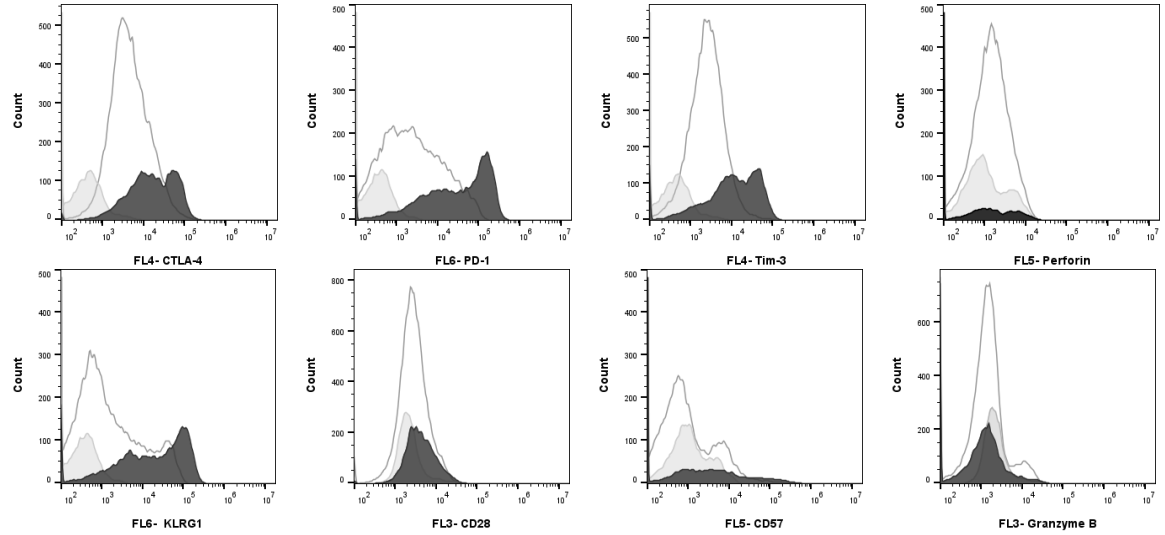
A: CIK + HubCCA1



B: CIK/mDC + HubCCA1



C: CIK/mDCSU + HubCCA1



Unstained CIK cells
Unexposed CIK cells
CCA-exposed CIK cells

Figure 4. Histogram of various markers on CIK cells under different conditions after HubCCA1 exposure. These conditions included untreated CIK cells (CIK + HubCCA1, A); mDC-pretreated CIK cells (CIK/mDC + HubCCA1, B); and sunitinib-primed mDCs-pretreated CIK cells (CIK/mDCSU + HubCCA1, C).

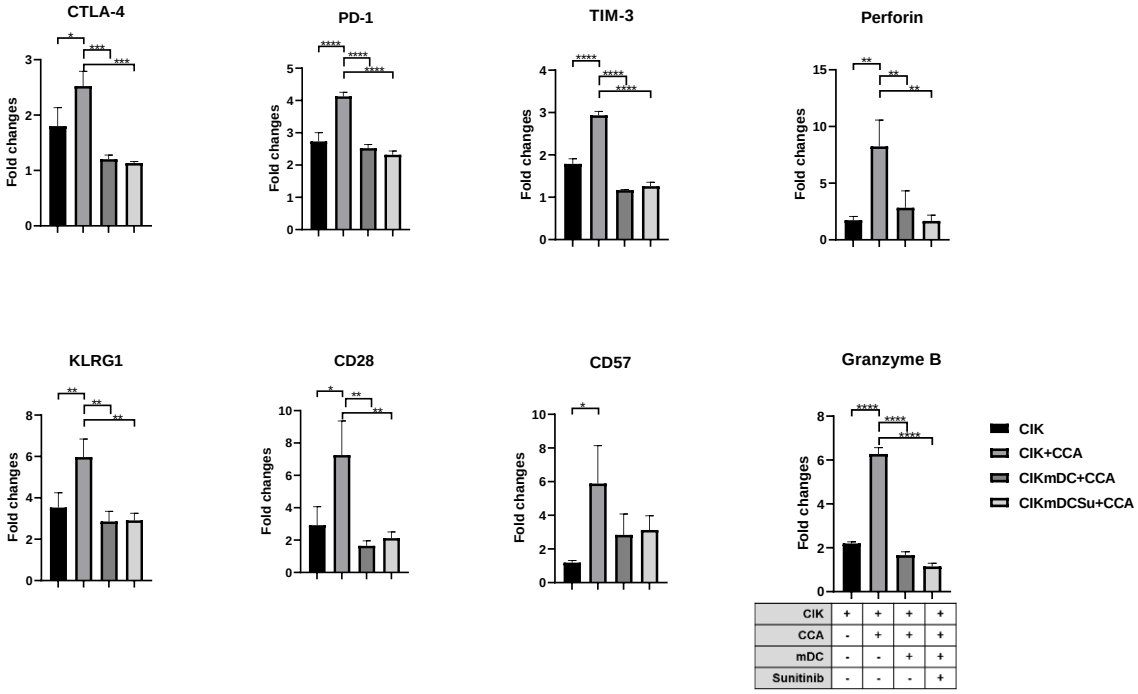


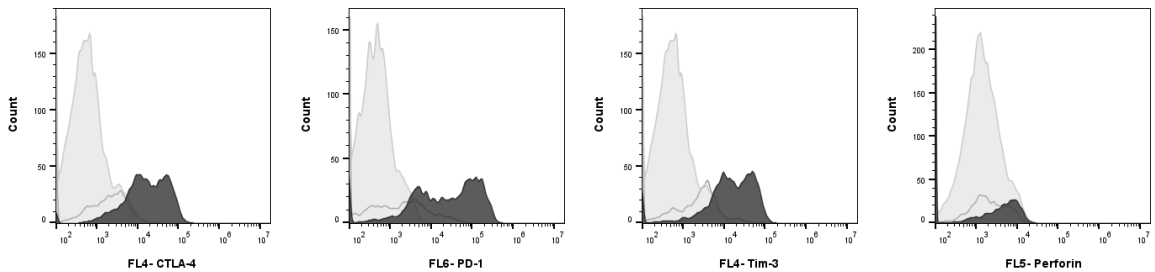
Figure 5. The phenotype of CIK cells was analyzed after 72-h HubCCA1 exposure. Normalized data were calculated, and statistical analysis was performed using one-way ANOVA followed by Tukey's test for multiple comparisons. Statistical significance was indicated as follows: * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$), and **** ($P < 0.0001$), with a 95% confidence interval.

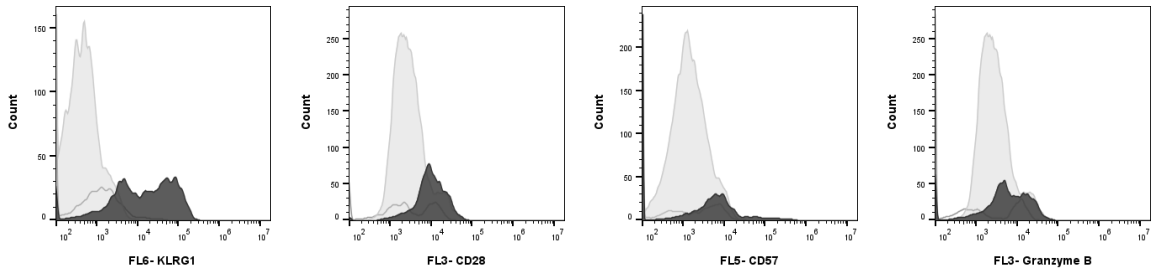
CIK pretreated with mDCs or sunitinib-primed mDCs partially prevented dysfunction of $CD3^+CD56^+$ subset from HubCCA1 exposure

The $CD3^+CD56^+$ subset was analyzed under the following conditions: $CD3^+CD56^+$, $CD3^+CD56^+$ + HubCCA1, mDC($CD3^+CD56^+$) + HubCCA1, and mDCSU($CD3^+CD56^+$) + HubCCA1 (**Figure 6**). This experiment demonstrated that the $CD3^+CD56^+$ subset in the $CD3^+CD56^+$ +CCA group showed a slight increase in the levels of exhaustion markers CTLA-4 ($p < 0.001$) and PD-1 ($p < 0.01$), and senescence markers KLRG1 ($p < 0.05$), with no significant changes observed in Tim-3, CD28, CD57, perforin, or granzyme B compared to the CIK group (**Figure 7**).

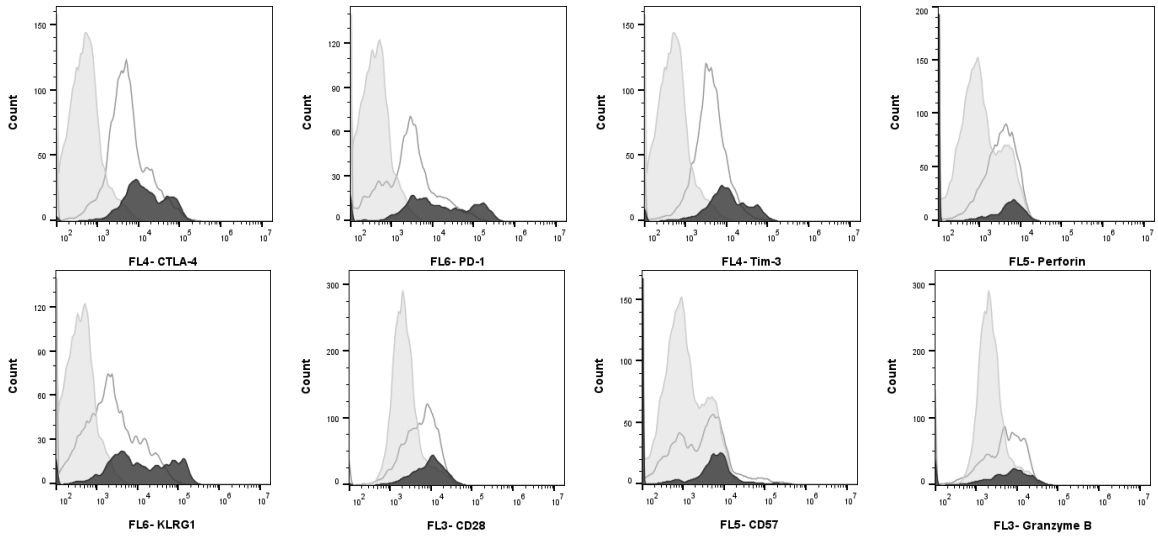
Pre-treatment of $CD3^+CD56^+$ cells with mDCs or sunitinib-primed mDCs (mDCSU) revealed that the treated $CD3^+CD56^+$ subset showed a significant reduction in CTLA-4. Notably, only the mDCSU($CD3^+CD56^+$) + HubCCA1 group showed a significant decrease in PD-1. No significant change was observed in KLRG1 level (**Figure 7**).

A: $CD3^+CD56^+$ subset + HubCCA1

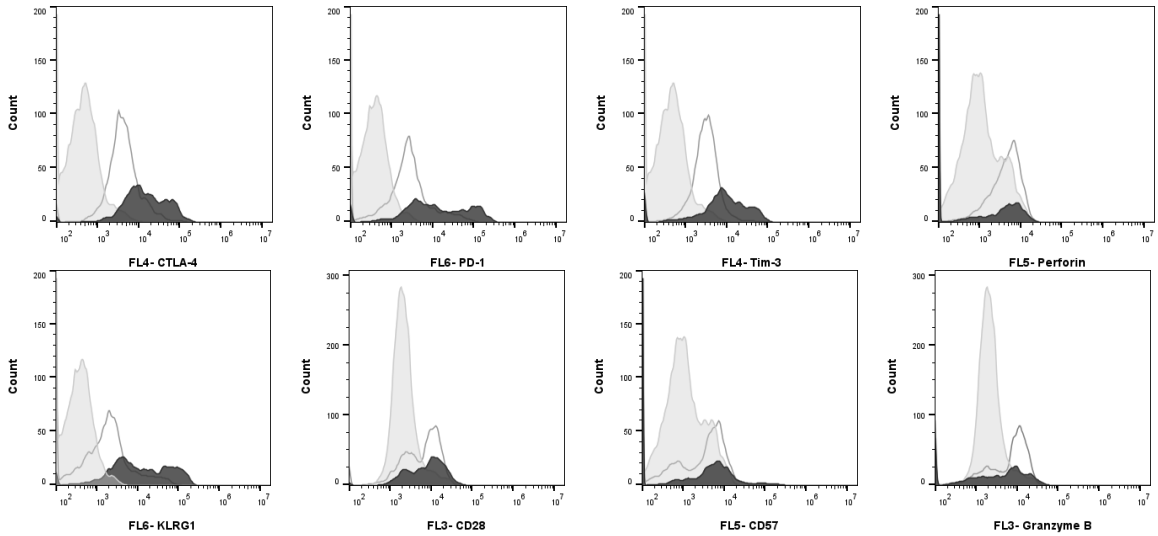




B: mDC($CD3^+CD56^+$) + HubCCA1



C: mDCSU($CD3^+CD56^+$)+ HubCCA1



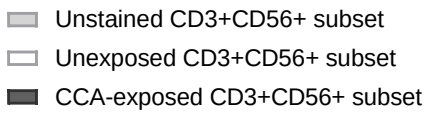


Figure 6. The histogram of various markers on $CD3^+CD56^+$ cells under different co- conditions after HubCCA1 exposure. These included untreated $CD3^+CD56^+$ cells ($CD3^+CD56^+$ + HubCCA1, A); mDCs pre-treated $CD3^+CD56^+$ cells (mDC($CD3^+CD56^+$) + HubCCA1, B); and sunitinib-primed mDCs pre-treated $CD3^+CD56^+$ cells (mDCSU($CD3^+CD56^+$) + HubCCA1, C).

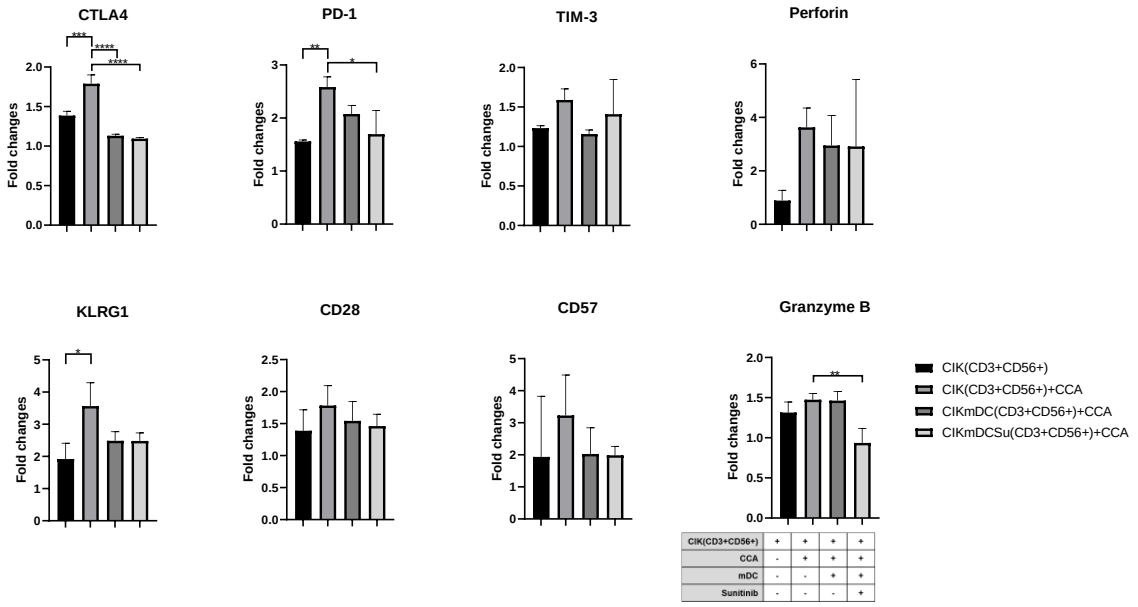


Figure 7. The phenotype of the $CD3^+CD56^+$ subset under different pre-treatment conditions was assessed after HubCCA1 exposure. Normalized data were calculated, and statistical analysis was performed using one-way ANOVA followed by Tukey's test for multiple comparisons. Statistical significance was indicated as follows: * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$), and **** ($P < 0.0001$), with a 95% confidence interval.

Discussion

PBMCs typically consist of 10-30% monocytes[12], while $CD3^+CD56^+$ cells generally represent around 1-5%[2], varying between individuals. $CD3^+CD56^+$ cells combine the properties of both T cells and natural killer (NK) cells, enabling them to target and destroy tumor cells[2]. $CD3^+CD56^+$ cells exhibited strong cytolytic activity against tumors. When activated, these cells released cytotoxic enzymes and cytokines, enhancing their tumor-killing potential.[10,13] For CIK cell generation, the $CD3^+CD56^+$ population typically consists of about 20-30%, peaking around day 20 before stabilizing. CIK cells have been considered a promising tool in cancer immunotherapy due to their potent cytotoxic effects and ability to kill without MHC restriction.[14] However, after prolonged exposure to tumor antigens, CIK cells may become exhausted and senescent.

Co-culture with HubCCA1 induced an exhausted/senescent phenotype in CIK cells, marked by increased exhaustion (CTLA-4, PD-1, Tim-3) and senescence (KLRG1, CD57) markers; however, cells also showed increased level of CD28 and effector markers (Perforin, Granzyme B). Despite reduced immune function, CIK cells retained some cytotoxic activity.[15-16] However, compared to total CIK cells, $CD3^+CD56^+$ subset showed fewer elevated markers after cancer exposure. Specifically, only CTLA-4, KLRG1, and PD-1 were significantly increased, indicating a more resistant phenotype to tumor-induced senescence.

There has been limited information regarding the downregulation of inhibitory receptors on $CD3^+CD56^+$ cells after stimulation. CCA exposure might trigger $CD3^+CD56^+$ cells, which were more sensitive than other subsets, toward cytotoxic activation as a rapid, innate-like response, thus downregulating inhibitory receptors like CTLA-4 and KLRG1 to preserve energy for cytotoxic functions. This would aim for a short-term burst of cytotoxicity against tumor cells at the cost of later exhaustion or functional decline.[17]

CD57 is a marker of more mature NK cells, which possesses higher levels of perforin and granzyme B compared to $CD57^-$ NK cells, but exhibits lower cytokine responsiveness and reduced proliferative capacity. $CD57^+$ NK cells have been shown to express slightly lower levels of KLRG1 compared to $CD57^-$ NK cells, suggesting that as NK cells mature, there may be a tendency to downregulate certain inhibitory receptors.[18]

T cell senescence in immune dysfunction is marked by telomere shortening, reduced telomerase activity, and increased levels of markers such as KLRG1 and CD57.[19-20] Effector T cells with $CD57^-KLRG1^+$ were less differentiated and had better proliferative capacity than did $CD57^+KLRG1^+$ cells.[21] KLRG1 was linked to reduced IFN-γ production and increased cell death in NK cells, with KLRG1 activation involving the AMP-activated protein kinase (AMPK) enzyme.[22] Additionally, CD28 and CD27 loss led to decreased production of key cytokines (IL-2, IFN-γ, TNF-α), compromising the immune response of senescent T cells.[23]

PD-1 is not solely a marker of T cell dysfunction.[24] It is an inhibitory receptor that functions through ITIM and ITSM motifs, which inhibit T cell proliferation and cytokine production.[25] LAG-3, CTLA-4, and other inhibitory receptors suppressed T cell function by interacting with MHC class II molecules, reducing TCR signaling, and contributing to exhaustion in chronic infections and cancer.[26] TIM-3, when co-expressed with other inhibitory receptors like PD-1, further inhibits T cell activation and cytokine production, promoting overall T cell exhaustion.[27]

Sunitinib has been shown to have significant immunomodulatory effects, particularly in the context of DC function and the reduction of immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs).[28] The combination of sunitinib with other immunotherapies enhances a more potent overall immune response.[29] CIK cells pre-treated with mDCs or sunitinib-primed mDCs showed reduced CTLA-4, PD-1, Tim-3, and KLRG1, along with decreased CD28, perforin, and granzyme B. Despite this, the presence of perforin and granzyme B suggested some preservation of effector function. This was consistent with the information that co-culturing CIK cells with mDCs enhanced CIK cell activation through the binding of CD80 and CD86 to CD28 on CIK cells, increasing cytokine production (such as IFN-γ) and anergy preventing.[30] This would resist exhaustion and immune suppression, allowing them to maintain their cytotoxic function in the tumor environment.[31] Moreover, this interaction promoted the differentiation of memory-like CIK cells, offering long-term immunity and potentially preventing tumor recurrence.[31]

This result showed that both CIK co-culture with mDC and CIK co-cultured with sunitinib-primed mDC conditions exhibited similar markers, with a slight difference observed only in the lessening of PD-1. CIK cytotoxicity increased after co-culture with mDCs and was highest when co-cultured with sunitinib-primed iDCs or mDCs, compared to untreated CIK cells.[11] Sunitinib-primed DCs enhanced the anti-tumor activity of CIK cells by inducing a Th1 phenotype and suppressing Th2/Th17 phenotypes, particularly in the $CD3^+CD56^+$ subset.[11, 32] Notably, immunoregulatory markers such as PD-L1 and IDO were decreased.[11]

Additionally, no harmful effect from sunitinib was observed, even at higher concentrations.[33-35] To enhance the effectiveness of cancer immunotherapy, further studies are needed to better understand and modulate CIK cells, their interactions with inhibitory receptors, and their relationships with other immune components.

Conclusion

CIK cells co-cultured with HubCCA1 (CIK+CCA) showed increased levels of inhibitory receptors associated with exhaustion and senescence, indicating impaired immune function and reduced anti-tumor activity. Pre-treatment with mDCs or sunitinib-primed

mDCs (CIKmDC+CCA and CIKmDCSU+CCA) significantly reduced these markers, helping preserve CIK cell function. Analysis focused on the $CD3^+CD56^+$ subset, which demonstrated greater resistance to tumor-induced exhaustion, with no significant upregulation of Tim-3, and CD57. Additionally, PD-1 showed a lower than CIK group. Notably, CTLA-4 level was reduced by both pre-treatments, while PD-1 reduction was observed only in the sunitinib-primed mDC group.

Co-culturing CIK cells with mDCs prevented exhaustion and suppression by the tumor microenvironment, enhancing their tumor-fighting ability and longevity, ultimately improving CIK cell-based cancer immunotherapies. However, outcomes depend on individual responses.

No conflicts of interest to declare

Acknowledgement

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Author's contribution

Nathawadee Sawatpiboon: Investigation (lead); methodology (equal); data collection (lead); formal analysis (lead); writing – original draft (lead) – review & editing (equal), Sunisa Duangsa-ard: investigation (supporting), Kanda Kasetsinsombat: Resources (lead), Adisak Wongkajornsilp: Conceptualization (lead); methodology (equal); validation (lead); funding acquisition (lead); supervision (lead); Writing – review & editing (equal)

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F04

Effects of the Dried Powder of Kai-Ka Min on a GERD Mouse Model with Limiting Food Access through Alternating Feeding and Fasting Conditions

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Abstract

Previous reports indicate that alternating feeding and fasting conditions can lead to overeating and gastric distension, which cause changes in the lower esophageal mucosa that contribute to the progression of gastroesophageal reflux disease (GERD). Controlling dietary patterns is a first-line approach for managing GERD symptoms. Kai-Ka Min (KK), a nodulose root of turmeric, has been suggested to relieve acid reflux symptoms, according to personal communication. However, no scientific report currently exists on the effects of KK in the treatment of GERD. This study investigates the effects of KK on GERD in a mouse model with limited food access through alternating feeding and fasting conditions. To induce GERD, female C57BL/6 mice were alternatively fed and fasted for 56 days and then treated with distilled water as the control, propylene glycol as the GERD model, 40 and 80 mg/kg body weight (BW) of KK, and 20 mg/kg BW of rabeprazole as the positive control, once daily for 6 weeks. Food intake was recorded throughout the experimental period. At the end of the experiment, gastric distension and histological examination of esophageal tissue were performed. In the model group with alternating feeding and fasting, a significant increase in both weekly and average food intake, as well as gastric distention ($p < 0.05$), was observed compared to the control group. Both of 40 and 80 mg/kg KK groups showed significant reductions in food intake and gastric distension ($p < 0.05$). The histological examination of the esophageal tissue revealed epithelial thinning, along with disorganization and exfoliation of the keratin layers. Interestingly, KK improved these histological alterations compared to the model group, similar to those in the control and rabeprazole-treated group. In conclusion, KK alleviated GERD induced by alternating feeding and fasting, plausibly by reducing food intake, gastric distension, and esophageal tissue damage, suggesting its potential as a therapeutic option for GERD.

Keywords: Gastroesophageal reflux disease, alternating feeding and fasting, overeating, Kai-Ka Min

Introduction

Gastroesophageal reflux disease (GERD) is an upper gastrointestinal (GI) disorder characterized by the backflow of gastric contents into the esophagus.[1] GERD is one of the

most common disorders, and its global prevalence has increased by 77.53%, from 441.57 million cases in 1990 to 783.95 million cases in 2019.[2] The typical symptoms of GERD include heartburn and regurgitation of acid reflux. Although the exact pathophysiology of GERD is poorly understood, it is though to involve impaired lower esophageal sphincter (LES) resting tone, transient LES relaxations (TLESRs), reduced esophageal acid clearance, and delayed gastric emptying.[3] Moreover, some studies have reported that several risk factors can trigger GERD, including excessive body mass index (BMI) and dietary controls that can lead to over food consumption, resulting in gastric distension and may be a plausible mechanism causing GERD.[4,5] However, GERD can lead to some complications such as reflux esophagitis, which results from esophageal injury by frequent reflux of gastric contents and is associated with inflammation, esophageal stricture, and an increased risk of developing Barrett's esophagus and then cause esophageal adenocarcinoma.[2,6]

Based on animal experimental studies of GERD models, previous models often required surgical procedures to induce reverse flow, allowing gastric or duodenal contents to reflux into the esophagus, but these models did not reflect physiological conditions, making it difficult to control the amount and concentration of the refluxed contents. However, recent studies suggest that GERD can be induced through various dietary controls, with the frequency of food consumption playing a critical role in triggering GERD. Additionally, excessive food consumption leads to gastric distension, which increases intragastric pressure and promotes the relaxation of the LES. This process facilitates acid reflux, resulting in esophageal mucosal damage and triggering an inflammatory response, which may contribute to the progression of GERD.

Currently, lifestyle modifications, such as dietary changes, body positioning including keeping the head elevated while sleeping and maintaining an upright posture for at least 2–3 hours after meals, and reducing body weight, serve as the first-line approach for managing GERD symptoms. Pharmacologic treatment primarily focuses on reducing gastric acid secretion through proton pump inhibitors (PPIs), which are considered first-line therapies for GERD.[7] However, there are certain limitations, including refractory symptoms, relapse, resistance, and adverse effects associated with long-term use.[8] Recently, there is increasing evidence to suggest the efficacy of focusing on the bioactive compounds of medicinal plants, which have been found to offer health benefits and alternative medicine in improving and treating GERD progression.[9]

Turmeric (*Curcuma longa* Linn.), a member of the Zingiberaceae family, has been utilized for centuries in traditional folk medicine in China, India, and Thailand to alleviate symptoms such as bloating, indigestion, diarrhea, and the treatment of stomach ulcers.[10,11] Its bioactive compound, particularly curcumin[10], a polyphenolic yellowish pigment, is known for its antioxidant, and anti-inflammatory activities, as well as its potential in promoting ulcer healing by reducing acid secretion and enhancing mucus production.[12] Furthermore, turmeric also contains components derived from adventitious roots, which are nodulose roots. These roots are round in shape and yellow in color, commonly known as "Kai-Ka Min" by local farmers in the southern part of Thailand. According to personal communications, the consumption of fresh Kai-Ka Min is believed to help alleviate symptoms of acid reflux and may be beneficial in the treatment of GERD. However, the pharmacological effects of Kai-Ka Min on GERD have not yet been scientifically investigated. Thus, this study aims to evaluate the effects of Kai-Ka Min on GERD, focusing on gastric distension and histological changes in a mouse model under time- and food-limited conditions through alternating feeding and fasting.

Methods

Kai-Ka Min (KK) preparation

The fresh KK root was obtained from the local market in the southern part of Thailand. Raw KK samples were washed, sliced to a 2 mm thickness and then lyophilized using a freeze-dryer. Dried KK was ground to be KK powder using blenders and the powder was stored at 4 °C until use.

Animals

Five-week-old female C57BL/6 mice, weighing 18-24 g were obtained from Nomura Siam International, Bangkok, Thailand. All mice were housed at the Laboratory Animal Service Center, Prince of Songkla University, Thailand. They were maintained in a temperature-conditioned room (25±2°C) with a 12-hour light-dark cycle and a relative humidity of 55±10%. Food and water were provided *ad libitum*. All mice were acclimatized to the laboratory conditions for one week before the experiment. The experiment was approved by the Institutional Animal Care and Use Committee of Prince of Songkla University, (MHESI 68014/704, Ref. AR045/2024).

Induction of GERD in experimental animals

Following one week of acclimatization, GERD was induced by limiting food access through alternately feeding and fasting for 56 consecutive days.[13] Distilled water (DW), propylene glycol (PG), and KK were administered daily by gavage, starting on day 14 of the experiment. All mice were randomly divided into five groups, each consisting of seven mice: Group 1 was the control group; mice were administered oral DW once daily with unlimited access to food and water. Group 2 was the model (GERD) group; mice with GERD were orally given PG once daily for 6 weeks. Groups 3 and 4 were the low- and high-dose of KK groups; mice with GERD were orally administered KK at doses of 40 and 80 mg/kg body weight (BW) orally once daily for 6 weeks, respectively. Group 5 was the Rabeprazole (Rb) group as a positive drug; mice with GERD were orally administered Rb at a dose of 20 mg/kg BW[14] once daily for 6 weeks (**Figure 1**). Throughout the experimental period, BW and food intake were recorded daily. At the end of the experiment, mice were anesthetized with thiopental sodium (100 mg/kg BW) via intraperitoneal injection. Subsequently, the esophagus and stomach were then collected for further analysis.

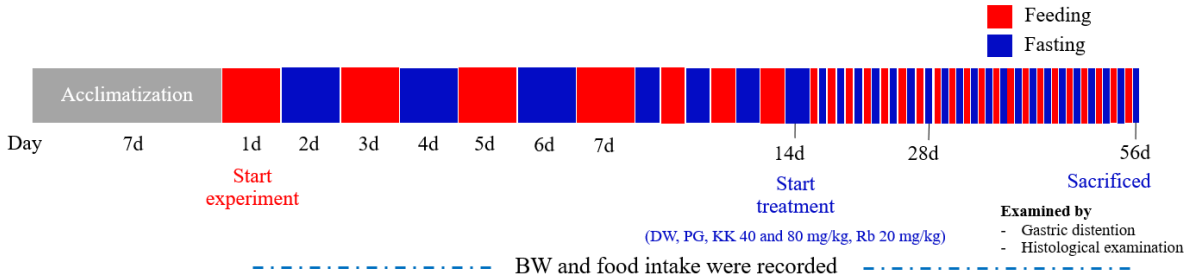

Figure 1. Schematic diagram of the experimental for a GERD mouse model with limiting food access through alternating feeding and fasting conditions (DW: Distilled water, PG: Propylene glycol, KK: Kai-Ka Min, Rb: Rabeprazole)

Gastric distention

After the mice were sacrificed, the stomach was carefully dissected and washed with ice-cold normal saline solution. The size of the stomach was then measured by its length, from the margin of the forestomach to the pylorus and its width, from the pylorus to the margin of the body of the stomach using a Vernier caliper and expressed as square millimeters (mm^2). The gastric distention was evaluated by measuring the differences in stomach size between experimental groups.

Histological examination

Following sacrifice, the esophageal tissues were immediately collected and fixed in 10% neutral-buffered formalin. The esophagus tissues were then dehydrated, cleared, and embedded in paraffin blocks. Paraffin-embedded esophageal tissues were cut into 5-µm-thick sections and stained with hematoxylin and eosin (H&E) for histological evaluation. Histopathological changes were examined under a light microscope at 40x magnification.

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using a one-way analysis of variance (ANOVA), followed by post-hoc Tukey's test using SPSS software. The results with $p < 0.05$ were considered statistically significant.

Results

Effect of KK on BW and food intake in a GERD mouse model with limiting food access through alternating feeding and fasting conditions

As previously mentioned, overconsumption can lead to gastric distension, contributing to GERD development. BW, weekly and average of food intake were measured for each group. The results showed that the average of BW did not differ significantly among the groups. Food intake in the control group remained consistent throughout the experiment. Compared to the control group, the model group exhibited a significant increase in both weekly food intake, observed from week 2 to week 8 of the experimental period, and average food intake (**Figure 2A-B**), confirming that limiting food access through alternating feeding and fasting in mice effectively developed a model for overeating, a potential trigger for GERD. Interestingly, both of 40 and 80 mg/kg KK groups, but not the Rb group, exhibited lower weekly and average food intake compared to the model group throughout the experimental period (**Figure 2A-B**).

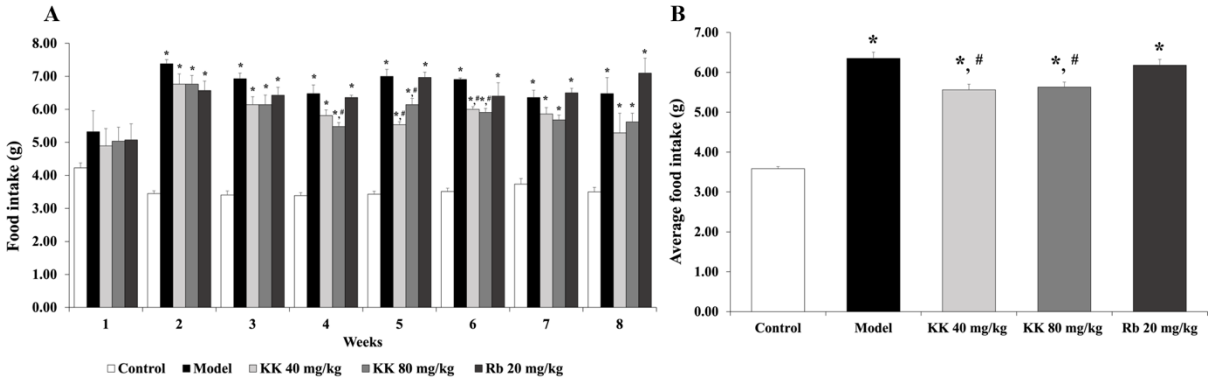

Figure 2. Effect of KK on food intake in a GERD mouse model with limiting food access through alternating feeding and fasting conditions. (A) weekly food intake and (B) average food intake in the five experimental groups. Data were presented as mean ± SEM ($n = 7$). $^{*}p < 0.05$ when compared with the control group and $^{\#}p < 0.05$ when compared with the model group, using one-way ANOVA with Tukey's post hoc test. KK: Kai-Ka Min, Rb: Rabeprazole.

Effect of KK on gastric distention in a GERD mouse model with limiting food access through alternating feeding and fasting conditions

At the end of the experiment, gastric distension was measured to evaluate its association with food intake (**Figure 3**). The model group exhibited significant gastric distention compared to the control group, suggesting that alternately feeding and fasting can cause overeating in mice, leading to increased gastric distention. Treatment with KK (40 and 80 mg/kg) and Rb administration resulted in a significant reduction in gastric distension, suggesting the potential efficacy of treatments in managing overeating and gastric distension.

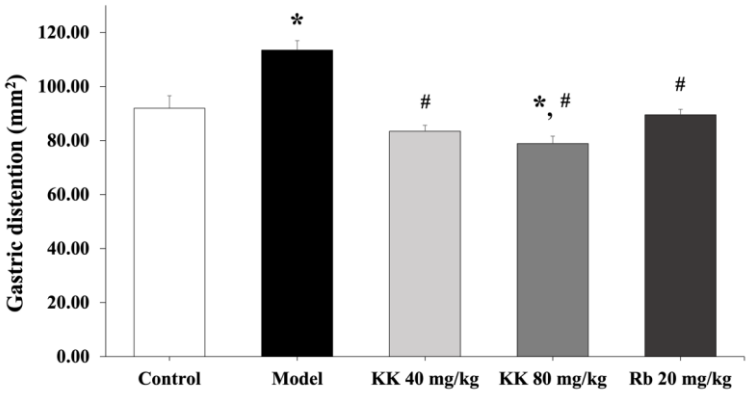

Figure 3. Gastric distention was assessed in each group at the end of the experiment. Data were presented as mean ± SEM (n = 7). $^{*}p < 0.05$ when compared with the control group and $^{\#}p < 0.05$ when compared with the model group, using one-way ANOVA with Tukey's post hoc test. KK: Kai-Ka Min, Rb: Rabeprazole.

Effect of KK on histological changes in a GERD mouse model with limiting food access through alternating feeding and fasting conditions

H&E-staining was performed to examine the histological changes in the esophageal tissues (**Figure 4**). In the control group, normal esophageal tissue characteristics were observed, including an intact esophageal epithelium and well-organized keratin layers. In the model group, the esophageal epithelium appeared thinner, with disorganized and exfoliated keratin layers. Alternatively, treatment with KK effectively improved these histological alterations.

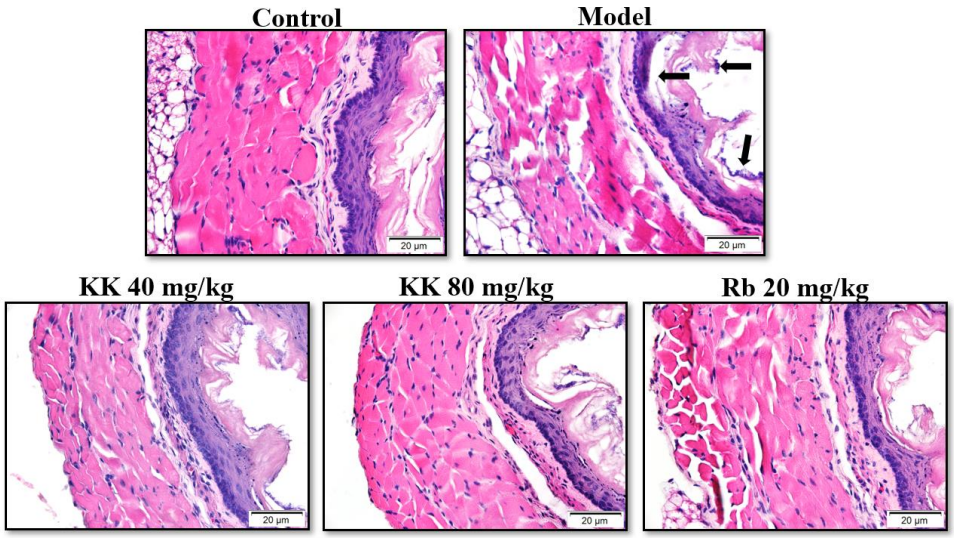

Figure 4. Histological examination of the esophageal tissues from each experimental group was conducted following alternating feeding and fasting conditions using H&E staining (n = 3). Images were captured

at a magnification of x40 with a scale bar of 20 μm, black arrow indicated the loss of epithelial cells. KK: Kai-Ka Min, Rb: Rabeprazole.

Discussion

The present study aims to evaluate the effects of KK on GERD in a mouse model under limited food access through alternating feeding and fasting conditions. Our results indicated that the mice with GERD exhibited increased weekly and average food intake, gastric distension, and esophageal tissue injury. Treatment with KK significantly reduced gastric distention, associated with a decrease in both weekly and average food intake in alternating feeding and fasting-induced GERD mice model. Furthermore, KK improved histological damage, including the esophageal epithelium and keratin layers of the esophageal tissue.

Evidence from scientific studies increasingly shows that the consumption of a large meals and dietary patterns such as alternating feeding and fasting conditions contribute overconsumption of food during feeding periods.[13] This factor further increases the amount of food in the stomach, leading to gastric distension, increased intra-abdominal pressure, and relaxation of the LES, all of which can contribute to the pathogenesis of GERD.[15] Furthermore, previous studies have reported a significant decrease in the pH value of gastric secretions in mice under alternating feeding and fasting conditions, which may lead to further injury to the esophageal lining and the development of reflux esophagitis and other complications of GERD.[16,17] The results of this study are consistent with these findings, showing a significant increase in both weekly and average food intake as well as an enlargement of gastric distension in the GERD-induced mice with limited food access under alternating feeding and fasting conditions. Treatment with KK resulted in a significant alleviation of these effects, as evidenced by a reduction in gastric distention and a decrease in both weekly and average food intake. Previous reports have proposed that turmeric contain bioactive compounds, primarily curcuminoids with curcumin as the main active component.[18] It has been shown to enhance digestion, reduce bloating and abdominal discomfort.[19] Additionally, curcumin affects GI peristalsis by promoting LES contraction and gastric emptying, which in turn reduced gastric distention and increased gastric pH.[12,19,20] Moreover, curcumin administration was associated with improvement in GI disorders including digestive symptoms, abdominal pain, and GERD.[12,22] These findings support the effectiveness of KK as a management strategy for GERD, especially in cases exacerbated by overeating and gastric distention.

An increase in food intake due to overeating can lead to gastric distention, which subsequently increases intra-abdominal pressure, relaxes the LES, and promotes the reflux of gastric contents into the esophagus. This reflux can trigger inflammation and further contribute to the development of esophageal injury and impairment of the esophageal mucosal barrier, which are key features of GERD.[16,17] Consistent with previous studies[13,16], histological analysis of the esophageal tissue of the mice with GERD under limited food access through alternating feeding and fasting conditions revealed esophageal lesions characterized by a thinning of the epithelial layer, the presence of cellular debris, and disorganization and exfoliation of the keratin layers. Interestingly, treatment with KK alleviated these histological changes and promoted recovery from esophageal injuries, with results comparable to those of the control and standard treatment group treated with rabeprazole. As mentioned, curcumin is the main bioactive compound of turmeric and has been reported to exhibit anti-inflammatory properties.[23] Previous studies have indicated that curcumin exerts gastroprotective effects in gastroesophageal disease by protecting against tissue damage, inhibiting the expression of inflammatory cytokines, reducing acid secretion, increasing mucus production, and alleviating gastric and esophageal injury in both human and animal models.[12,23,24] These results suggest that the bioactive compound found in the KK has

therapeutic effects, potentially protecting against inflammation and esophageal tissue damage associated with GERD.

Conclusion

In conclusion, this study demonstrates that KK is effective in alleviating gastric distension, reducing food intake, and improving esophageal tissue injury in a mouse model of GERD induced by alternating feeding and fasting. These results suggest that KK may be a promising nutraceutical for managing GERD, particularly when exacerbated by dietary patterns and gastric distention. However, further studies are needed to explore the underlying mechanisms through which KK exerts these protective effects, including identifying the active compounds, its impact on inflammatory pathways, and evaluating its long-term efficacy to fully establish its therapeutic potential.

Acknowledgment

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F05

Sangyod Rice Extract Reduces Lipid Accumulation in Adipose Tissue of Streptozotocin-Induced Diabetic Rats

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Abstract

Recently, Sangyod rice extract (SREE) has been reported to inhibit adipocyte differentiation *in vitro*. However, its *in vivo* effect on visceral fats has not yet been reported. This study examined the effects of SREE on adipogenic gene expression and lipid metabolism in perirenal and epididymal fat tissue of type 2 diabetic (T2DM) rats. Five groups of male Sprague-Dawley rats (n=7) were assigned as follows: normal control (NC) group, T2DM control (DM), T2DM treated with the standard drug metformin 200 mg/kg (DMM200), and T2DM treated with SREE 250 mg/kg (DMS250) or SREE 500 mg/kg (DMS500). The result demonstrated that DMM200, DMS250, and DMS500 significantly upregulated the expression of major adipogenic transcription factors, such as peroxisome proliferator-activated receptor gamma (PPAR-γ) and CCAAT/enhancer-binding protein (C/EBP)-α (C/EBPα) in visceral fat compared to the DM group. The mRNA expression level of C/EBPβ was reduced in DMM200, and DMS500, while the C/EBPγ level showed a significant reduction only in DMM200 group compared to the DM group. Moreover, high-dose SREE and metformin treatments significantly reduced mRNA levels of glucose transporter 4 (Glut4), insulin receptor (InSR), fatty acid synthase (FASN), and adipose triglyceride lipase (ATGL) compared with the DM group. However, no significant difference in Glut4, FASN, and ATGL expressions were observed between DMS250 and DM groups. In conclusion, these findings suggest that SREE holds promise as a therapeutic approach for managing adipose tissue function in T2DM.

Keywords: Sangyod rice, type 2 diabetic, perirenal fat, epididymal fat, adipogenesis, lipid metabolism

Introduction

Type 2 diabetes mellitus (T2DM), a multifactorial metabolic disorder, poses a significant global health crisis characterized by tissue insulin resistance, impaired insulin secretion, and inadequate compensatory insulin response.[1] The development of T2DM in relation with high-fat diets (HFDs) consumption contributes to adipocyte hypertrophy and lipid metabolism dysfunction, exacerbating insulin resistance and T2DM. The disruption of systemic lipid homeostasis promoting adipogenesis via lipogenic genes such as peroxisome proliferator-activated receptor gamma (PPAR-γ) and CCAAT/enhancer binding protein

(C/EBP) isoforms (α, β, and γ). PPAR-γ, a key regulator of adipocyte differentiation, induces the formation of lipid droplet and upregulates lipogenic enzymes. Concurrently, C/EBPα stabilizes PPAR-γ, while C/EBPβ and C/EBPγ initiate adipogenesis by stimulating PPAR-γ during early differentiation.[1,2] Lipid metabolism is governed by enzymes including adipose triglyceride lipase (ATGL), which hydrolyzes triglycerides into free fatty acids, and glucose transporter protein (Glut4), is known to facilitate insulin-stimulated glucose uptake through insulin receptor (InSR) signaling. Previous studies reported that the impairment of Glut4 translocation and InSR dysfunction cause insulin resistance, while reduced ATGL activity and increased the expression of fatty acid synthase (FASN), contributing to lipid accumulation.[3]

Metformin is widely recognized as the first-line medication for managing type II diabetes because of its proven effectiveness, favorable safety profile, good tolerability, and minimal risk of hypoglycemia.[4-6] Recent studies have demonstrated that metformin can prevent weight gain and the accumulation of fat mass in obese mice fed a high-fat diet.[7,8]

Sangyod rice, a red-to-violet pigmented rice, contains high levels of phenolic compounds and flavonoid.[9] and possess various pharmacological activities, such as antihypertension[10], modulation of spatial learning and memory[11], inhibition of adipocyte growth in 3T3-L1 cells[9], and amelioration of insulin resistance in hepatic cells *in vitro*.[12] However, the potential of Sangyod rice extract (SREE) in ameliorating adipose dysfunction *in vivo* T2DM models remains unexplored. Therefore, this study investigated whether SREE modulates adipogenesis and lipid metabolism in T2DM rats.

Methods

Animal experimentation

Male 5-week-old Sprague-Dawley rats were sourced from Nomura Siam International, Thailand. They were housed under standard conditions, with a temperature of 22 ± 1°C and a 12-hour light-dark cycle. The rats had ad libitum access to food and water. Following a one-week acclimatization period, experimental procedures approved by the Animal Ethics Committee of Naresuan University (NU-AE650710). The rats were randomly into two dietary regimens: one group was fed a normal diet, and the other a high-fat diet (HFD) as previously described by Hanchang et al.[12] Following three weeks of dietary intervention, the rats on the HFD were received an intraperitoneal injection of streptozotocin (STZ) at 35 mg/kg body weight, whereas the rats on the normal diet group were injected with the vehicle (citrate buffer). Five days post-STZ injection, rats with fasting blood glucose concentrations ≥200 mg/dL were classified as T2DM models and selected for further experiments. The diabetic rats were then divided into five groups, each containing seven rats: Group I, the Normal control group (NC); Group II, the Diabetic control group (DM); Group III, the Diabetic rats treated with 200 mg/kg body weight of metformin (DMM200); Group IV, the Diabetic rats treated with 250 mg/kg body weight of SREE (DMS250); and Group V, the Diabetic rats treated with 500 mg/kg body weight of SREE (DMS500). SREE was extracted, solubilized in 50% polyethylene glycol, and administered orally to the respective groups, as described in the previous study by Hanchang et al.[12] During the 30-day treatment period, fasting blood glucose, body weight, and food and water intake were monitored regularly.[7] After that, the rats were

fasted overnight and euthanized with thiopental sodium at a dose of 100 mg/kg body weight. Perirenal fat and epididymal fat tissues were collected for further analysis.

Histology

Perirenal and epididymal adipose tissues were fixed with freshly prepared in 4% paraformaldehyde (Sigma-Aldrich) and embedded in paraffin wax. The 5-µm-thick sections were obtained using a rotatory microtome (Leica, Germany) and rehydrated through a graded ethanol series at concentrations of 100%, 95%, and 75%, respectively. The tissues sections were subsequently stained with Masson's trichrome. Histological alterations were examined by a DP73 light microscope (Olympus, Tokyo, Japan).

Analysis of mRNA expression by real-time PCR

Total RNA was extracted from perirenal and epididymal adipose tissues using TRIzol reagent (Invitrogen Life Technologies), following optimized protocols tailored for lipid-rich tissues. cDNA synthesis was performed using the Superscript VILO cDNA synthesis kit (Thermo Fisher Scientific Inc., Waltham, MA, USA). Quantitative PCR analysis was conducted using the SensiFAST SYBR NO-ROX kit (Thermo Fisher Scientific) on a BIOER real-time PCR detection system (Hangzhou Bioer Technology, China) to quantify mRNA expression of PPAR-γ, C/EBPα, C/EBPβ, C/EBPγ, Glut4, InSR, FASN, and ATGL, with β-actin serving as the endogenous control. The primer sequences and amplification protocol were previously described by Woonnoi et al.[9] Relative mRNA expression was determined by the $2^{-\Delta\Delta CT}$ method, with β-actin expression used for normalization.

Statistical analysis

All data are presented as mean values ± standard error of the mean. One-way ANOVA was conducted to identify variances among multiple groups, followed by the Duncan post hoc test. Statistical significance was determined at a threshold of $P < 0.05$.

Results

Effects of SREE on perirenal fat and epididymal fat weight in T2DM rats

The results showed that the weights of perirenal fat and epididymal fat in the DM group did not differ significantly from those in the NC group. Additionally, there were no significant differences in the weights of the DMM200, DMS250, and DMS500 groups compared to the DM group (**Figure 1**).

Effects of SREE on histological changes in adipose tissue of T2DM rats

Histological findings revealed that the adipose tissue of diabetic rats exhibited hypertrophy, characterized by enlarged size of fat cells, and increased fibrosis, as demonstrated by the blue-green staining of collagen bundles around blood vessels and adipocytes, compared to the adipose tissue of NC rats. In contrast, the DMM200, DMS250, and DMS500 groups showed less pronounced alterations in adipose tissue architectures compared to the DM group (**Figure 2**).

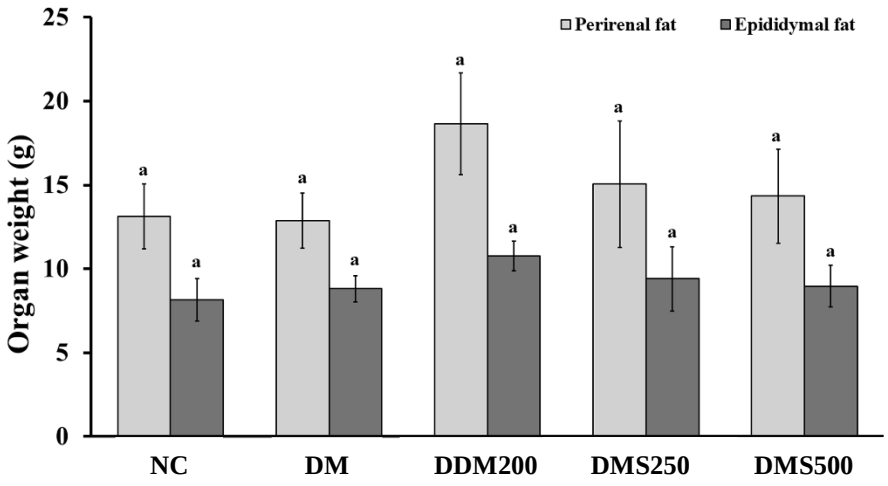

Figure 1. Effects of SREE on perirenal fat and epididymal fat weight in T2DM rats. All values are presented as mean ± SEM, with n = 7 per group. The groups are defined as follows: **NC:** Normal control rats, receiving a normal diet and 50% polyethylene glycol, **DM:** Diabetic rats, receiving HFD and 50% polyethylene glycol, **DMM200:** Diabetic rats, receiving HFD and 200 mg/kg body weight (BW) of metformin, **DMS250:** Diabetic rats, receiving HFD and 250 mg/kg BW of SREE, and **DMS500:** Diabetic rats, receiving HFD and 500 mg/kg BW of SREE

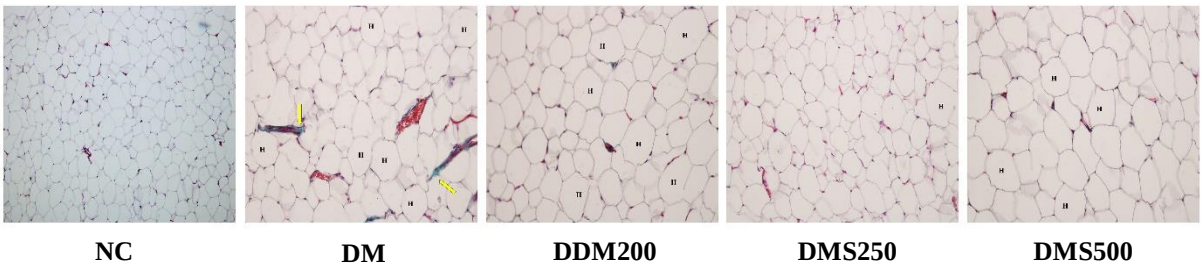

Figure 2. Effects of SREE on histological changes in adipose tissue of T2DM rats. The groups are defined as follows: **NC:** Normal control rats, receiving a normal diet and 50% polyethylene glycol, **DM:** Diabetic rats, receiving HFD and 50% polyethylene glycol, **DMM200:** Diabetic rats, receiving HFD and 200 mg/kg BW of metformin, **DMS250:** Diabetic rats, receiving HFD and 250 mg/kg BW of SREE, and **DMS500:** Diabetic rats, receiving HFD and 500 mg/kg BW of SREE. Scale bar = 40 µm

Effects of SREE on the expression of adipogenic gene in adipose tissue of T2DM rats

We assessed the mRNA levels of key adipogenic genes, including PPAR-γ, C/EBPα, C/EBPβ, and C/EBPγ. Notably, the expression of PPAR-γ and C/EBPα was significantly decreased in the DM group compared to NC group. In contrast, the DMM200, DMS250, and DMS500 groups showed a significant increase in PPAR-γ and C/EBPα compared to the DM group (**Figure 3A-B**). Regarding C/EBPβ, its expression was elevated in the DM group compared to the NC group. However, this increase was significantly attenuated in the DMM200, and DMS500 groups compared to the DM group. No significant difference was observed for DMS250 compared to the DM group (**Figure 3C**). For C/EBPγ, the gene expression was higher in the DM group than in the NC group. The DMM200 group showed a significant decrease in C/EBPγ expression compared to the DM group. In contrast, no

significant changes were observed in the DMS250 and DMS500 group relative to the DM group (**Figure 3D**).

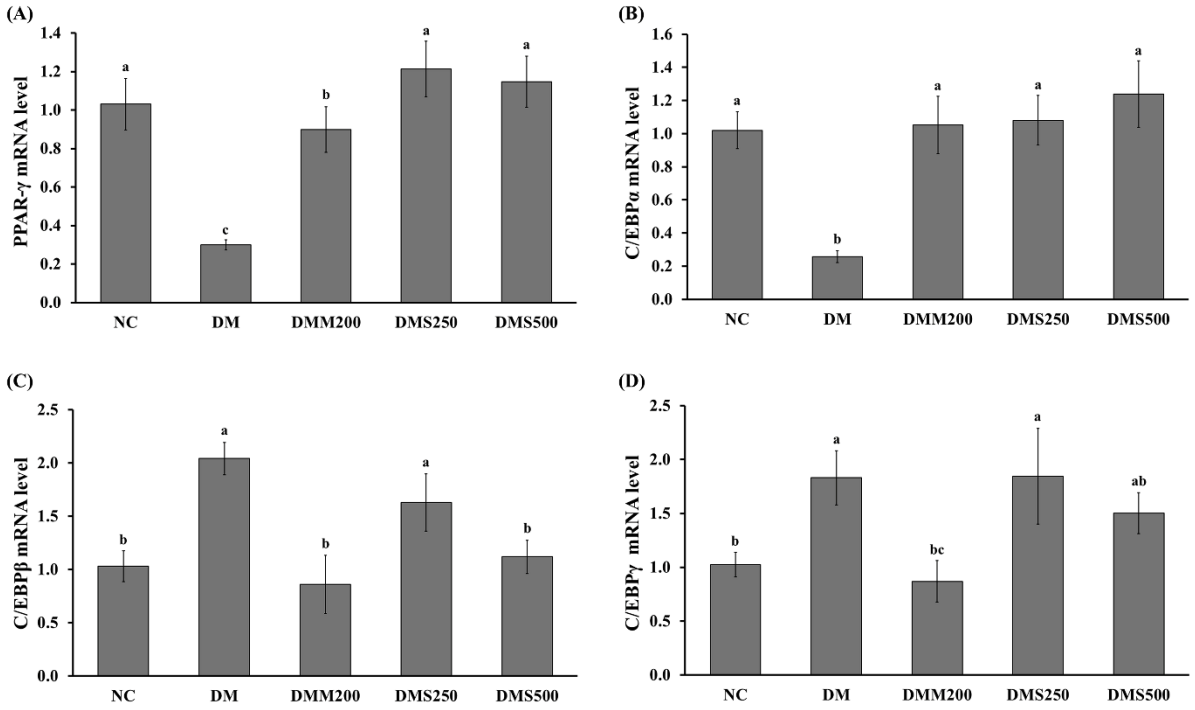

Figure 3. Effects of SREE on the expression of adipogenic transcription factors genes in adipose tissue of T2DM rats. All values are presented as mean ± SEM, with n = 7 per group. Different lowercase letters denote statistical significance ($p < 0.05$). The groups are defined as follows: **NC:** Normal control rats, receiving a normal diet and 50% polyethylene glycol, **DM:** Diabetic rats, receiving HFD and 50% polyethylene glycol, **DMM200:** Diabetic rats, receiving HFD and 200 mg/kg BW of metformin, **DMS250:** Diabetic rats, receiving HFD and 250 mg/kg BW of SREE, and **DMS500:** Diabetic rats, receiving HFD and 500 mg/kg BW of SREE

Effects of SREE on the gene expression of lipid metabolism enzyme in adipose tissue of T2DM rats

We evaluated the expression of key enzymes involved in lipid metabolism, including *Glut4, InSR, FASN*, and *ATGL* (**Figure 4A-D**). The expression of *InSR* was significantly upregulated in the DM group compared with the NC group. However, the DMM200 and DMS500 groups exhibited a significant decrease in *InSR* expression compared with the DM group (**Figure 4A**). The expression of *Glut4* was significantly elevated in the DM group compared with the NC group. In contrast, DMM200 and DMS500 treatments resulted in a significant reduction in *Glut4* expression compared with the DM group, whereas DMS250 did not differ significantly from the DM group (**Figure 4B**). Additionally, both *FASN* and *ATGL* gene expressions were significantly upregulated in the DM group. In response to treatment, the DMM200 and DMS500 groups showed a significant decrease in *FASN* and *ATGL* expression compared with the DM group. However, DMS250 did not exhibit a significant change in FASN and ATGL expression compared with the DM group (**Figure 4C, D**).

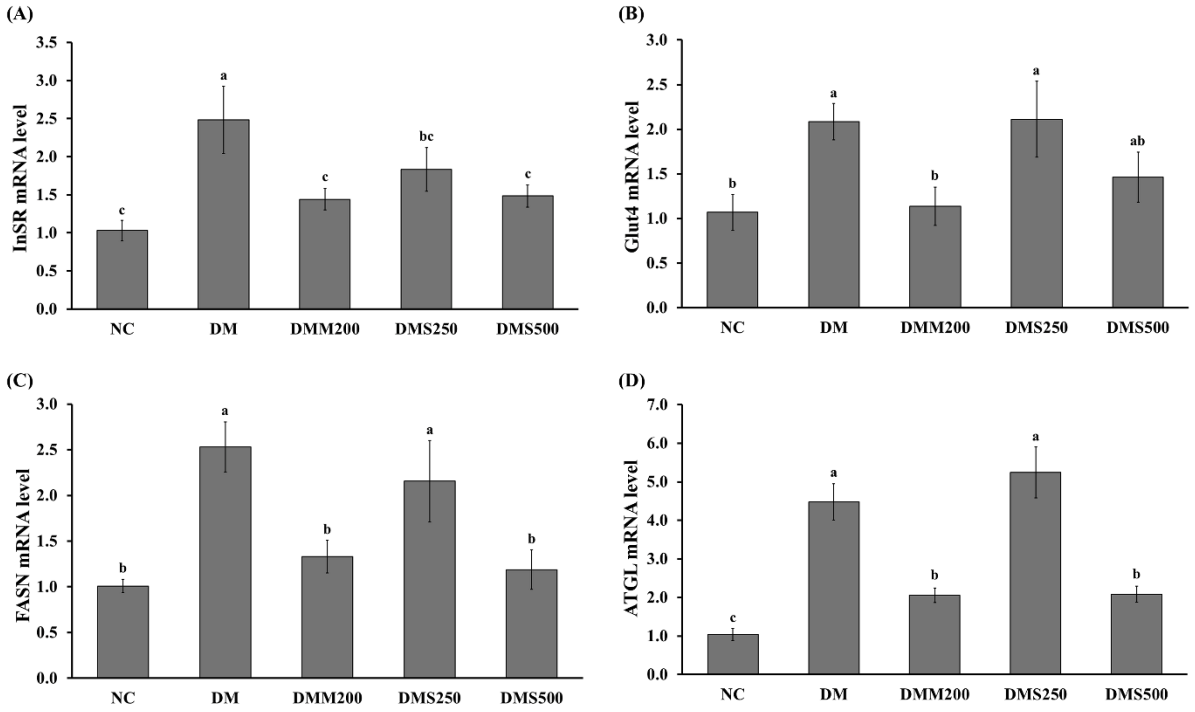

Figure 4. Effects of SREE on the gene expression of lipid metabolism enzyme in adipose tissues of T2DM rats. All values are presented as mean ± SEM, with n = 7 per group. Different lowercase letters denote statistical significance ($p < 0.05$). The groups are defined as follows: **NC:** Normal control rats, receiving a normal diet and 50% polyethylene glycol, **DM:** Diabetic rats, receiving HFD and 50% polyethylene glycol, **DMM200:** Diabetic rats, receiving HFD and 200 mg/kg BW of metformin, **DMS250:** Diabetic rats, receiving HFD and 250 mg/kg BW of SREE, and **DMS500:** Diabetic rats, receiving HFD and 500 mg/kg BW of SREE

Discussion

Our previous studies demonstrated that SREE mitigates insulin resistance in hepatic cells and reduces hepatic steatosis in diabetic rats through mechanisms involving adenosine monophosphate-activated protein kinase (AMPK), mechanistic target of rapamycin (mTOR), and mitogen-activated protein kinase (MAPK) signaling pathways.[12] Additionally, SREE reduced the proliferation of 3T3-L1 cells during the early phase of adipocyte differentiation by downregulating the expression of mTOR and Akt, and it also inhibited lipid accumulation in adipocytes.[9] However, its effects on visceral adipose tissues, which play a critical role in systemic insulin sensitivity and lipid homeostasis, remain unexplored. In this study, we investigated the impact of SREE on adipogenesis and lipid metabolism in perirenal and epididymal adipose tissue of T2DM rats.

Our findings provided a significant insight into histology and gene expression of two primary visceral fat depositions including perirenal and epididymal fats from T2DM rats. The observed upregulation of PPAR-γ and C/EBPα expression in visceral fats by SREE consistent with their role as crucial regulators of adipocyte differentiation and lipid storage. PPAR-γ activation promotes adipogenesis, redirecting lipid deposition to adipose tissue and reducing the accumulation of ectopic fat in organs. C/EBPα sustains PPAR-γ expression and directly upregulates InSR, enhancing adipose insulin sensitivity.[13] SREE's modulation of C/EBPβ/γ aligns

with their role in initiating adipogenesis by priming PPARγ expression, particularly under diabetic stress. Our previous study reported that Sangyod rice extract inhibits adipocyte differentiation in 3T3-L1 adipocytes by suppressing key adipogenic transcription factors PPAR-γ, C/EBPα, C/EBPβ, sterol regulatory element-binding protein (SREBP) -1c (SREBP-1c), SREBP-2.[9]

Our study revealed significant alterations in the expression of key lipid metabolism enzymes in the perirenal and epididymal fat tissues of T2DM rats treated with SREE. The increased expression of *Glut4* and *InSR* in the DM group compared to the NC group reflects compensatory mechanisms to enhance glucose uptake and insulin signaling under diabetic conditions. However, treatment with DMM200 and DMS500 reduced *Glut4* and *InSR* expression, suggesting improved glucose utilization and reduced metabolic stress in adipose tissues. These results are consistent with those of Woonnoi et al.[9], who reported that Sangyod rice extract inhibits glucose uptake by suppressing the expression of Glut4 and InSR mRNA in 3T3-L1 adipocytes. Similarly, elevated *FASN* and *ATGL* levels in the DM group indicate heightened lipogenesis and lipolysis, characteristic of disrupted lipid metabolism in diabetes. SREE treatment, particularly at higher doses (DMS500), significantly decreased *FASN* and *ATGL* expression, reflecting its role in restoring lipid homeostasis by modulating adipocyte lipid synthesis and breakdown.[14,15] These findings were consistent with previous studies showing that improved lipid metabolism reduces ectopic fat deposition and lipotoxicity, mitigating insulin resistance.[16] However, the absence of significant changes in lipid metabolism enzymes such as Glut4, FASN, and ATGL in the DMS250 group suggests that the lower dose of SREE may not be sufficient to induce substantial alterations in these pathways.

Conclusion

This study demonstrates that SREE modulates adipogenesis and improving lipid metabolism in perirenal and epididymal fat deposition of T2DM rats. The upregulation of key adipogenic transcription factors, including PPAR-γ and three isoforms of C/EBP and modulation of lipid metabolism enzymes suggest that SREE may mitigate metabolic dysfunctions associated with T2DM on adipose tissue function. These findings provide an insight into the therapeutic potential of SREE in managing T2DM. Future research should explore the translational relevance of these effects to human T2DM.

Acknowledgement

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F06

Effects of *Spatholobus Littoralis* Hassk Root Ethanolic Extract on Anxiety-like Behaviors in Mice

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Abstract

Anxiety disorders are among the most prevalent mental health conditions worldwide, significantly impacting daily life and well-being. *Spatholobus littoralis* Hask., a medicinal plant rich in flavonoids, polyphenols, and other bioactive compounds, has been traditionally used in herbal medicine and is hypothesized to exert anxiolytic effects. However, empirical evidence supporting these effects remains limited. This study aimed to evaluate the effects of *Spatholobus littoralis* Hask. ethanolic extract (BE) on anxiety-like behavior in mice. Male outbred Mlac: ICR mice were randomly assigned to four groups and forced fed with distilled water (0.05 mL/kg body weight), fluoxetine (20 mg/kg body weight), BE 100 mg/kg body weight, or BE 200 mg/kg body weight for 14 days. Anxiety-like behaviors were assessed using the elevated plus maze (EPM) and light-dark box (LDB) tests on days 1, 7, and 14. The results demonstrated that no significant differences in body weight were observed among the mice groups treated with BE. However, the administration of BE 200 mg/kg significantly increased the time spent in the open arms of the EPM and the light compartment of the LDB, as well as the total number of entries and crossings, compared to control (distilled water) and other treatment groups on day 14. These findings suggest that BE 200 may exert anxiolytic effects.

Keywords: Anxiety, *Spatholobus littoralis* Hask, elevated plus maze, light-dark box, anxiolytic effects

Introduction

Anxiety disorders have been increasingly prevalent over the past three decades, affecting approximately 4.05% of the global population, or 301 million individuals, with a rise of over 55% from 1990 to 2019.[1] In fact, anxiety is a natural response to perceived threats; however, when symptoms become excessive, persistent, and disruptive to daily life, it is classified as generalized anxiety disorder (GAD), characterized by physical symptoms such as dizziness, nausea, and increased heart rate, alongside emotional disturbances like persistent worry, social withdrawal, and intrusive thoughts, ultimately impairing cognitive function.[2] Suicidal ideation demonstrates a significant association with anxiety disorders, as over 70% of individuals with a history of suicide attempts have experienced anxiety, which can develop due to stress, underlying medical conditions such as diabetes, comorbid depression, genetic susceptibility, childhood trauma, and substance use disorders.[3-5]

The underlying pathophysiology of anxiety disorders is complex, involving dysregulation of neurotransmitter systems, including serotonin, norepinephrine, and gamma-

aminobutyric acid (GABA) and abnormalities in brain regions such as the amygdala and prefrontal.[6-8] Therefore, these complex mechanisms highlight the challenges involved in fully understanding and treating anxiety disorders. Recent studies have shown that conventional drugs with anxiolytic and antidepressant effects, such as benzodiazepines (BZs; e.g., diazepam), selective serotonin reuptake inhibitors (SSRIs; e.g., fluoxetine), and selective norepinephrine reuptake inhibitors (SNRIs; e.g., venlafaxine), are commonly used to treat anxiety, with alternatives available including buspirone, pregabalin, and moclobemide.[9] However, these treatments are associated with adverse effects, including motor dysfunctions, sleep disturbances, pharmacological dependence, abuse potential, and sexual dysfunction.[10,11] Consequently, alternative treatments for anxiety disorders using natural products like plant-based therapies are gaining attention due to their antioxidant, anti-inflammatory and neuroprotective properties which can reduce the symptoms of GAD.[12] Furthermore, natural products have been reported to exhibit more efficacy than side effects.[13]

Spatholobus littoralis Hassk., commonly known as Bajakah Tampala, is a medicinal plant traditionally used in Southeast Asia, particularly in Indonesia, to treat conditions such as fever, diarrhea, and joint pain, and it contains bioactive compounds like flavonoids, saponins, and phenolic acids, with its root extract showing a notably high flavonoid content of 59.38%.[14-16] Although some studies highlight its medicinal properties, its potential effects on neurological conditions, particularly anxiety disorders, remain largely unexplored. Hence investigating its potential as a natural treatment for anxiety could provide valuable insights into alternative therapeutic approaches.

Behavioral tests are commonly used to assess the effects of both acute and chronic stress on brain functions, behaviors, and neurochemical changes, while also aiding in understanding anxiety disorders through mechanisms like neurotransmitter dysregulation, neuroinflammation, HPA axis alterations, and neuroplasticity.[17,18] The elevated plus maze (EPM) and light-dark box (LDB) tests are commonly used, where increased time in open arms (EPM) or the light compartment (LDB) indicates reduced anxiety.[9,19,20] We hypothesized that administration of *Spatholobus littoralis* Hassk. ethanolic extract (BE) would exert significant anxiolytic effects in mice, as evidenced by increased time spent in the open arms of the EPM and the light compartment of the LDB test – both established indicators of reduced anxiety-like behavior. Therefore, this study aimed to evaluate the potential of BE in anxiety-like behaviors in mice, thereby supporting its candidacy as a natural therapeutic agent for anxiety disorders.

Methods

Spatholobus littoralis Hassk ethanolic extract

Bajakah Tampala (*Spatholobus Littoralis* Hassk) roots were obtained from Andalas Medicinal Plants store, Banjarmasin, South Kalimantan, Indonesia and extracted at the Department of Pharmacology Laboratory, Faculty of Medicine, Khon Kaen University, Thailand. Bajakah ethanolic extract (BE) was produced by soaking 2kg of the sawdust in vessels with (1:3) 70% ethanol and covered tightly for three days. Subsequently, it was stirred and closed again tightly. It was filtered using many layers of gauze to obtain the filtrate. Re-maceration of the pulp was carried out using 70% ethanol. The filtrate was concentrated and dried using a rotary evaporator and freeze dryer. The dried powdered extract was eventually collected and weighed.

Experimental animals

Male outbred Mlac: ICR mice, aged between 6-8 weeks, and weighing between 25-35 grams were procured from the Northeast Laboratory Animal Centre (NELAC), Khon Kaen,

Thailand and fed – food (obtained from NELAC) and water ad libitum, under controlled conditions of a 12-h dark/light phase (lights on from 06:00–18:00) under 54% relative humidity at 20.2 °C. The mice received unlimited access to food and water for 14 days. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Khon Kaen University, based on the Ethic of Animal Experimentation of National Research Council of Thailand on 25 October 2024. Record No. IACUC-KKU-113/67, Reference No. 660201.2.11/822 (120).

Grouping and treatments

Mice were randomly divided into 4 groups consisting of 5 mice in each group and treated differently. The mice were forced-fed via oral gavage with distilled water (DW) 0.05ml/10g body weight/day (Group 1) as a control group, fluoxetine (as positive control) at 20mg/kg body weight/day (Group 2), low dose of BE, 100mg/kg body weight/day (Group 3), high dose of BE, 200mg/kg body weight/day (Group 4) and during treatment all mice were kept in their home cages located in the housing room. The experimental groups and treatments are illustrated in **Figure 1**.

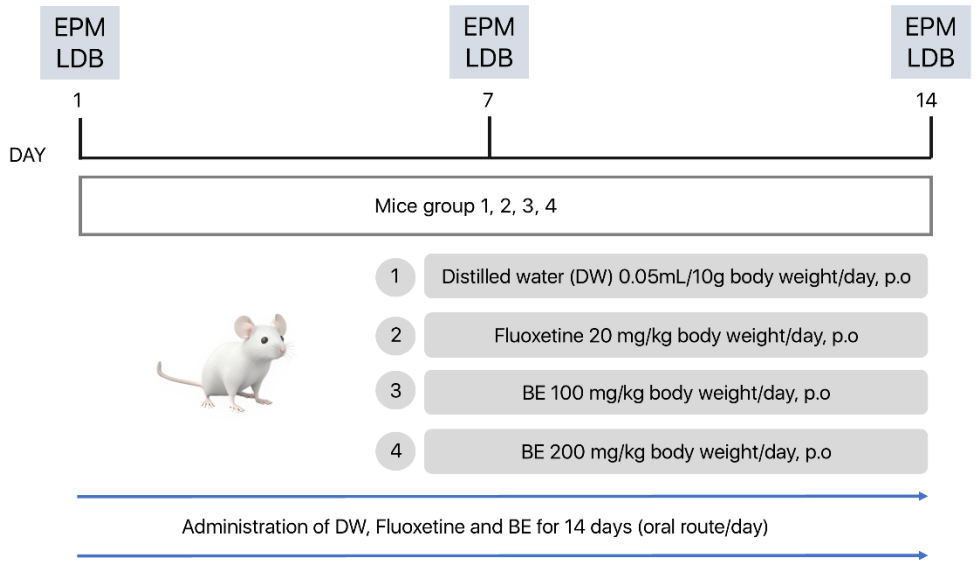

Figure 1. Experimental design of the study

Behavioral tests

Elevated plus maze (EPM) test

The EPM test is known as the main method for assessing anxiety-related behaviors in rodents. A plus-shaped apparatus with two open arms (30 x10 cm) without any wall, and two enclosed arms of the same size with 5 cm high side walls and end wall, and the central arena (10 x 10 cm) interconnecting all the arms were used in this experiment. The maze was raised from the ground by 60 cm. This test was conducted by placing the mouse in the central arena with its head directed towards one of the enclosed arms and allowed to explore freely for a duration of 5 minutes. A camera was used to record the activity of each mouse.[21,22]

Light dark box (LDB) test

The LDB test is also a well-known and widely used method for evaluating anxiety-like behavior in rodents. The testing apparatus, a plexiglass box with two compartments: a safe

small dark compartment, taking up one-third of the box (dimensions: W40 x L20 x H20 cm), and the remaining two-thirds of the box which was lighted by a 60-Watt white incandescent bulb (W40 x L20 x H20 cm) and a small opening door (dimensions: W8 x H12 cm) connecting the two compartments (W20 x H20), were used in this experiment. The mice were positioned in the center of the light area, facing the dark side. The following behaviours were noted and recorded by using a camera during a 5-minute observation period: (1) the duration of time spent in the light compartment (with all four legs), and (2) the number of crossings from the dark area to the light area.[21,23]

Toxicity tests

During treatment, mice were weighed daily and abnormal behaviors, if existed, were recorded. All mice were euthanized using the cervical dislocation technique and the blood was withdrawn via cardiac puncture method to measure alanine aminotransferase (ALT) and creatinine levels at the clinical chemistry laboratory unit of Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, using UV kinetic enzymatic method for ALT and Jaffe colorimetric method for creatinine.

Statistical analysis

All data were expressed as mean ± SEM and analyzed by one-way ANOVA followed by Tukey's multiple comparison post-hoc test. The comparison between 2 groups was analyzed using a two-tailed student t-test. All analyses were carried out using the GraphPad Prism version 8.01 for Windows (San Diego, California, USA). Results were considered significant at $p<0.05$.

Results

The effects of Spatholobus Littoralis Hassk ethanolic extract (BE) on body weight

Daily monitoring of mice over a 14-day period revealed distinct patterns in body weight changes across different treatment groups. Mice treated with fluoxetine showed a temporary weight loss on Day 3 (38.40 ± 2.205 g) and Day 4 (38.60 ± 2.206 g), followed by gradual recovery from Day 5 (39.60 ± 1.720 g) onward. In contrast, mice receiving BE 100 and BE 200 showed no significant weight differences compared to the control group (distilled water). However, their body weights were significantly different from the fluoxetine-treated group (41.20 ± 1.020 g for BE 100; 43.40 ± 1.030 g for BE 200). After Day 7, body weights in all groups stabilized, suggesting an adaptation period as shown in **Figure 2**. These findings indicate that BE extract does not cause weight loss, unlike fluoxetine, making it a potentially safer option in terms of body weight regulation. In addition, no abnormal behaviors or any signs of discomfort could be observed.

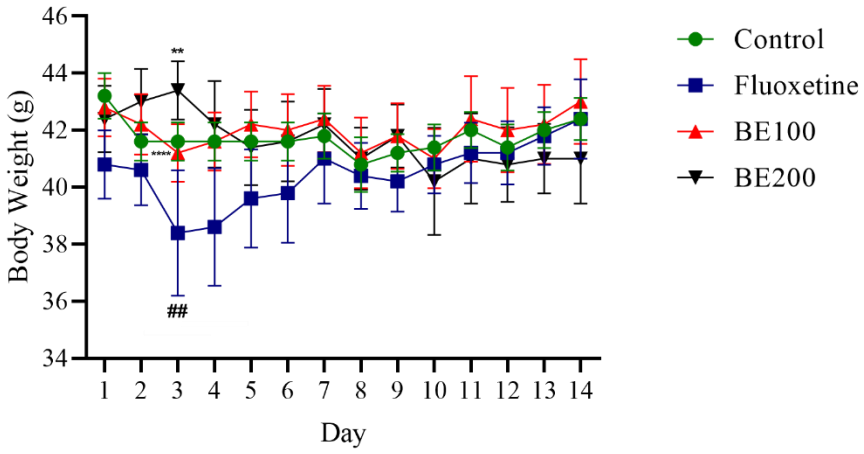

Figure 2. The effect of BE on body weight. Data represent mean (g) ± SEM (n = 5/group). ##p < 0.01, when compared to control group (distilled water); **p < 0.01 and ****p < 0.0001 when compared to positive control group (Fluoxetine); one-way ANOVA with Tukey's multiple comparisons post-hoc test.

The effects of BE on the time spent in open arms (s) and total number of entries in EPM

The administration of BE exhibited notable anxiolytic effects in the EPM test, as indicated by the time spent in the open arms (s) (**Figure 3A**) and the total number of entries (**Figure 3B**). On Day 1, BE 200 (30.50 ± 3.227 s) showed a significantly lower time spent in the open arms compared to both the control group (52.50 ± 0.866 s) and the positive control (fluoxetine; 0.25 ± 5.105 s), indicating that anxiolytic effects had not yet developed. By Day 7, all treatment groups (fluoxetine, BE 100, and BE 200) exhibited an increase in time spent in the open arms compared to the control group, with mean ± SEM for BE 100 (54.33 ± 1.764 s) and BE 200 (46.50 ± 7.365 s). Among them, fluoxetine showed the highest increase 85.00 ± 6.083 s). In addition, both BE 100 and BE 200 demonstrated significant differences when compared to the positive control. However, on Day 14, only the BE 200 group (41.40 ± 3.501 s) maintained a significant increase in time spent in the open arms compared to both the control (17.00 ± 2.121 s) and the positive control (24.00 ± 2.887 s).

With regards to the total number of entries, no significant differences were observed among any groups on Days 1 and 7. However, by Day 14, only the BE 200 group (17.80 ± 1.463) exhibited a significantly higher number of entries compared to the positive control (12.250 ± 0.479), even though no significant difference was found compared to the control group. These findings suggest that while fluoxetine showed the most immediate anxiolytic effect by Day 7, BE 200 demonstrated a progressive and sustained effect, becoming more pronounced by Day 14. Furthermore, the total number of entries in the EPM test signifies that the mice remained active during the experiment.

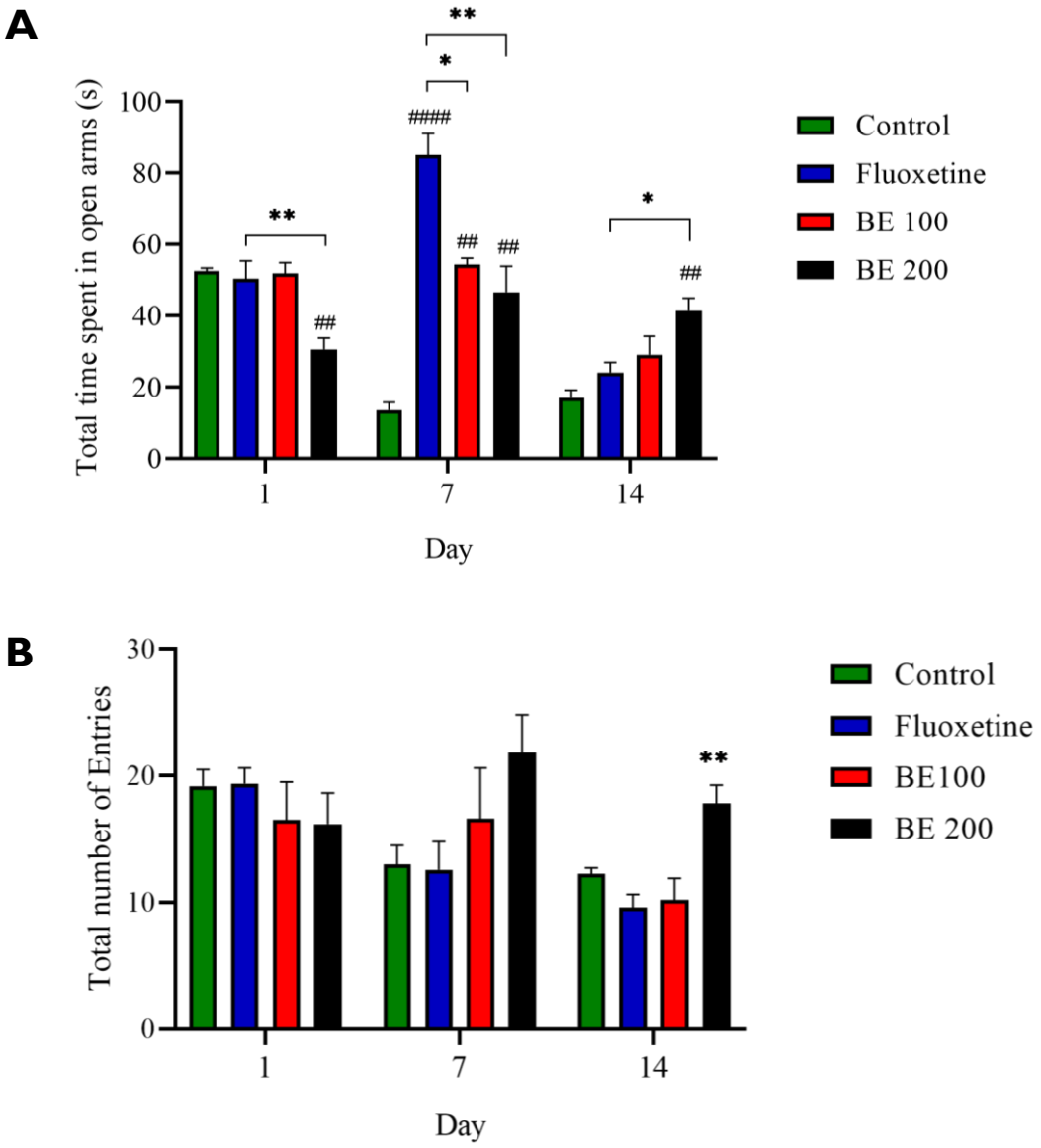

Figure 3. The effect of BE on **A)** the time spent in open arms (s), **B)** total number of entries in EPM. Data represent mean ± SEM (n = 5/group). ##p < 0.01 and #####p < 0.0001 when compared to control group (distilled water); *p < 0.05 and **p < 0.01 when compared to positive control group (Fluoxetine); one-way ANOVA with Tukey's multiple comparisons post-hoc test. The comparison between two groups was analyzed using a two-tailed student t-test.

The effects of BE on the time spent in the light compartment (s) and the number of crossings from the dark area to the light area in LDB

In this study, the effect of BE on anxiety-like behaviour was also evaluated using the LDB test across three different time points – Day 1, Day 7, and Day 14, as indicated by the time spent in the light compartment (s) (**Figure 4A**) and the total number of crossings from the dark area to the light area (**Figure 4B**). On Day 1, there were no significant differences among all groups, except for the mice treated with BE 200 (7.50 ± 0.500 s), which showed a slight anxiolytic effect compared to the control group (5.00 ± 1.00 s). By Day 7, the BE 200 group (109.00 ± 21.00 s) exhibited the highest time spent in the light compartment (s) compared to all other groups, including the control group (2.33 ± 0.882 s) and the positive control group treated with Fluoxetine (3.50 ± 0.500 s). On Day 14, when compared to the

control group (2.00 ± 0.577 s), all treatment groups (Fluoxetine 20, BE 100, and BE 200) showed significant differences (19.00 ± 1.528 s for Fluoxetine, 56.66 ± 1.764 s for BE 100 and 73.66 ± 2.66 for BE 200). Furthermore, the BE 100 and BE 200 groups spent significantly more time in the light compartment compared to the positive control group, suggesting that BE 200 has a considerable anxiolytic effect.

The number of crossings into the light compartment in LDB test provides insights into the anxiolytic effects of BE over 1, 7, and 14 days of treatment. Furthermore, it also indicates that the mice were still active during the experiment. On Day 1, there was no significant difference between the control and treatment groups, indicating that the anxiolytic effects might be time-dependent. By Day 7, when compared to the control group (1.75 ± 0.750), the BE 200 treatment group showed a significant increase in the number of crossings (10.50 ± 2.500), demonstrating the most pronounced effect. Moreover, there was also a significant difference between the positive control (1.33 ± 0.33) and the BE 200 group. The anxiolytic effects were more evident by Day 14 where both the BE 100 (8.66 ± 0.88) and BE 200 groups (10.00 ± 1.826) showed a significantly increased number of crossings compared to the control group (1.00 ± 0.00) and the positive control (2.75 ± 0.479). However, there was no significant difference between the mice group treated with fluoxetine and the control group. Therefore, these results suggest that BE has an anxiolytic effect over time, particularly BE 200.

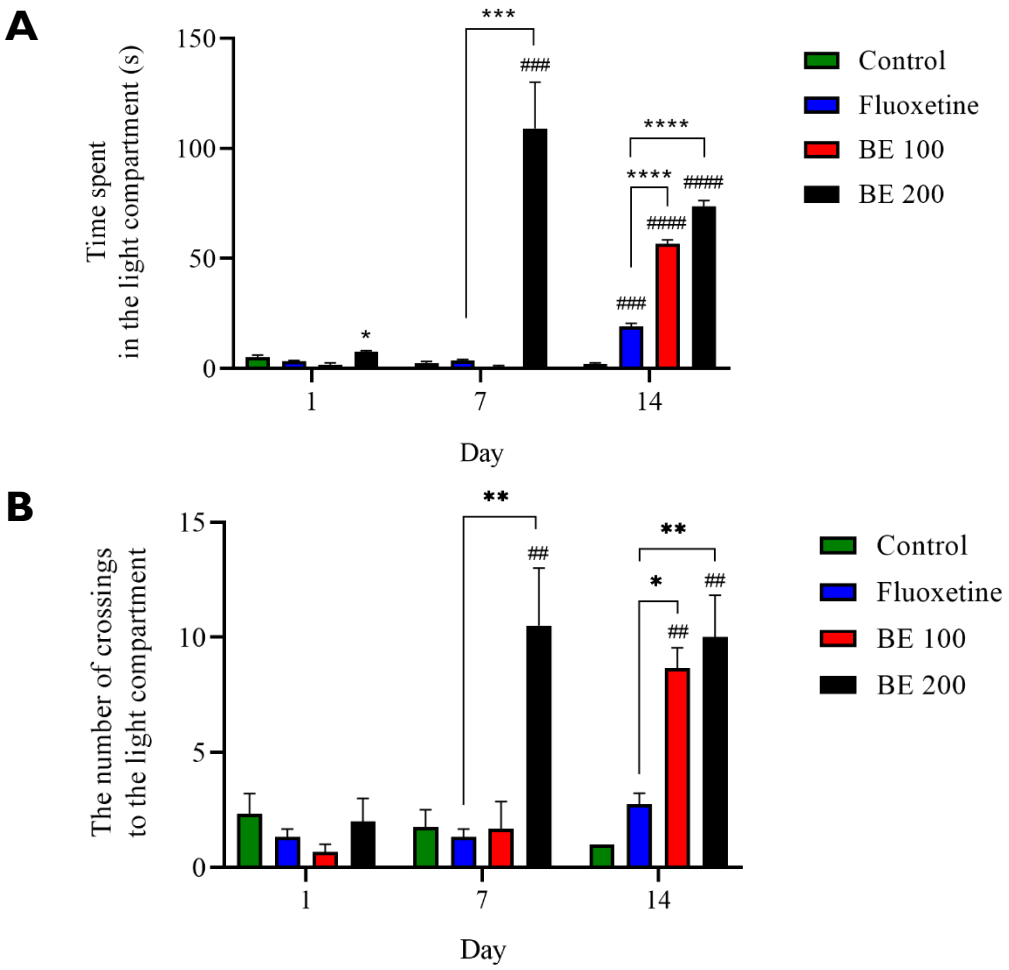

Figure 4. The effect of BE on **A)** the time spent in the light compartment (s), **B)** the number of crossings from the dark area to the light area in LDB. Data represent mean ± SEM (n = 5/group). #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ and #####$p < 0.0001$ when compared to control group (distilled water);

*p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.001 when compared to positive control group (Fluoxetine); one-way ANOVA with Tukey's multiple comparisons post-hoc test. The comparison between two groups was analyzed using a two-tailed student t-test.

The effects of BE on ALT and creatinine Levels

The effects of BE on ALT levels (**Figure 5A**) and creatinine levels (**Figure 5B**) were evaluated to assess potential hepatic and renal toxicity. In this study, there were no statistically significant differences in ALT levels among the treatment groups (fluoxetine; 293.667 ± 27.425 U/L, BE 100; 284.250 ± 50.878 U/L, and BE 200; 67.400 ± 16.842 U/L) compared to the control group (213.750 ± 85.387 U/L), indicating no evidence of hepatic toxicity. Similarly, creatinine levels did not differ significantly between any of the treatment groups (fluoxetine; 0.360 ± 0.063 µg/dL, BE 100; 0.390 ± 0.052 µg/dL, and BE 200; 0.282 ± 0.045 µg/dL) and control group (0.280 ± 0.050 µg/dL), suggesting no evidence of renal toxicity. These findings suggest that administration of BE at both 100 mg/kg and 200 mg/kg does not induce detectable liver or kidney damage under the experimental conditions. The lack of change in ALT supports the hepatic safety of BE, while stable creatinine levels indicate preserved renal function. Furthermore, the absence of toxicity in the fluoxetine-treated group aligns with its known safety profile at the administered dose. Overall, the results imply that BE is unlikely to exert adverse effects on liver or kidney function at the tested dosages.

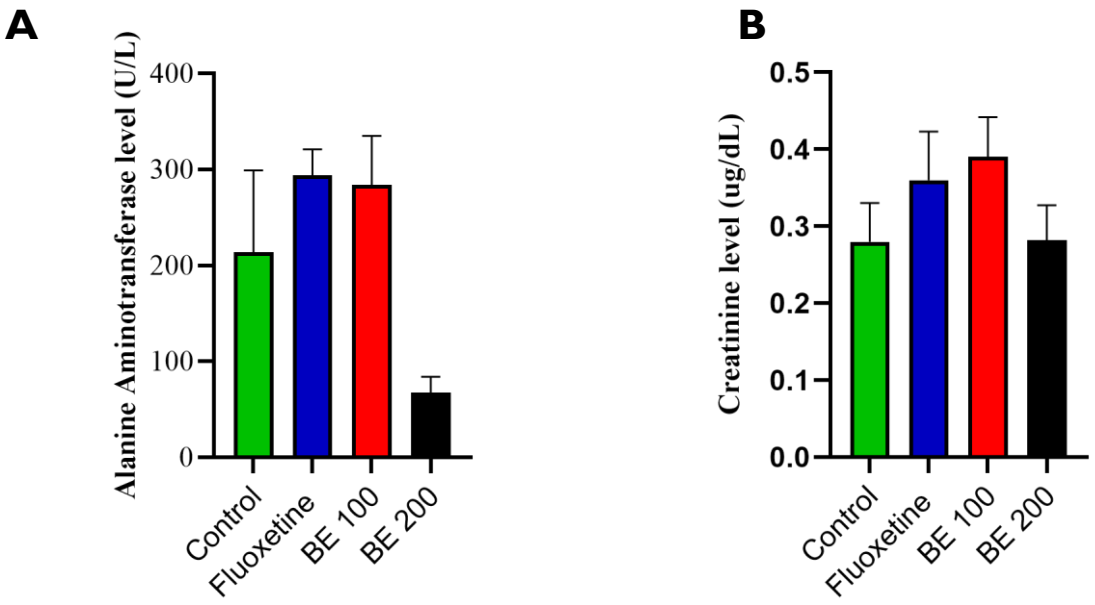

Figure 5. The effect of BE on **A)** ALT level (U/L), **B)** Creatinine level (µg/dL). Data represent mean ± SEM (n = 5/group). One-way ANOVA with Tukey's multiple comparisons post-hoc test was used and the comparison between two groups was analyzed using a two-tailed student t-test. The p < 0.05 is considered as significantly statistical difference.

Discussion

Spatholobus Littoralis Hassk ethanolic extract (BE) contains several bioactive compounds, including flavonoids, terpenoids, saponins, tannins, and phenolic compounds

which are known for their antioxidant and anti-inflammatory properties.[15,24,25] These compounds, particularly flavonoids have been shown to interact with neurotransmitter systems such as gamma-aminobutyric acid (GABA), serotonin, and dopamine, which are crucial for mood regulation and anxiety responses.[26-28] The presence of these bioactive molecules suggests that BE might be able to exert anxiolytic effects.

For a plant-based compound to exhibit anxiolytic properties, it must possess the ability to modulate the central nervous system, particularly the neurotransmitter systems involved in anxiety regulation.[29] Compounds with antioxidant and anti-inflammatory properties are often beneficial in reducing anxiety, as both acute and chronic stress, as well as anxiety, have been linked to oxidative damage and neuroinflammation.[30-33] Additionally, a plant extract must be bioavailable and capable of crossing the blood-brain barrier (BBB) to effectively interact with brain receptors.[34,35] Flavonoids exert neuroprotective effects on the central nervous system, thereby offering therapeutic potential for neurodegenerative diseases, including mood disorders. [36,37] The presence of bioactive molecules like flavonoids in BE suggests that it meets these criteria, making it a viable candidate for further investigation as an anxiolytic agent. Furthermore, low toxicity levels of plant-based extracts are essential in drug development to ensure safety and tolerability.[38] In this study, the levels of ALT, a liver enzyme used as a biomarker for hepatocellular injury,[39] and creatinine, a waste product indicative of kidney function, were measured to assess potential hepatic and renal toxicity.[40] Elevated ALT levels may indicate liver damage, while increased creatinine levels suggest impaired renal function. [41,42] However, there were no statistically significant differences in ALT or creatinine levels between the treatment groups (fluoxetine, BE 100, and BE 200) and the control group, indicating the absence of liver or kidney toxicity. Notably, the observed ALT and creatinine levels remained within a low and physiologically normal range, supporting the conclusion that *BE* is well-tolerated at the tested doses. In addition, during 14 days of treatment, all mice were normally alive and no abnormal behaviors and/or any signs of abnormalities could be observed. These findings further reinforce the potential of *BE* as a safe and promising anxiolytic candidate for future therapeutic applications.

In the body weight analysis, treatment with BE did not result in any significant changes in the mice body weight, indicating that it does not have weight-altering effects. This stability in body weight suggests that BE may offer a safer alternative to synthetic anxiolytics unlike fluoxetine, which caused a temporary reduction in body weight. The weight loss induced by fluoxetine may be attributed to its serotonergic activity, which influences appetite and metabolism. In both mice and humans, fluoxetine can suppress appetite, leading to weight reduction.[43-45] In contrast, BE does not appear to interfere with metabolic pathways to the same extent, indicating that it may be less likely to cause side effects such as weight loss, making it a preferable option for long-term use.

The EPM and LDB tests are widely used to assess anxiety-like behaviour in animal models because they rely on natural conflict responses. The EPM test measures the animal's preference for the open arms (associated with fear and anxiety) versus the closed arms (which provide safety), making it a reliable tool to evaluate anxiety.[46] Similarly, the LDB test exploits the natural aversion of animals to bright light, with increased exploration of the light compartment indicating reduced anxiety. Both tests are essential for assessing anxiolytic effects because they mimic the internal conflict between fear and exploration.[47,48] In this study, BE treatment resulted in a progressive increase in the time spent in the open arms of the EPM and the light compartment of the LDB, reflecting a decrease in anxiety. The greater number of crossings and entries further supports the anxiolytic potential of BE, likely due to its neurochemical modulation, which encouraged more exploration and reduced avoidance behaviour.[49]

The overall results of this study suggest that BE has a significant anxiolytic effect, particularly at the higher dose, as evidenced by increased time spent in the open arms of the EPM and the light compartment of the LDB, as well as a higher number of entries and crossings. This effect was sustained over time, with BE 200 showing a more pronounced anxiolytic effect by Day 14, in contrast to fluoxetine, which exhibited a more immediate but shorter-lasting effect. Furthermore, fluoxetine treatment did not significantly alter the number of crossings in the LDB test. The previous study also reported no significant differences in light/dark transitions (total number of crossings) following repeated fluoxetine treatment (at doses of 10 mg/kg and 20 mg/kg) in mice. This lack of effect may be attributed to the habituation process whereby mice become more accustomed to the testing environment, exhibit reduced exploratory behavior and greater passivity.[50] These findings indicate that BE may act as a gradual and sustained anxiolytic, with its effectiveness increasing over time. Some anxiolytic drugs, including plant-based compounds like BE, can have a longer onset of action due to the time required for them to accumulate in the body, interact with receptors, and produce therapeutic effects.[28] In contrast to fast-acting drugs, which often provide immediate relief through direct neurotransmitter modulation, slower-onset compounds may work by gradually altering neurotransmitter balance or reducing neuroinflammation, leading to more sustained, long-term benefits.[51] This delay in onset can also be a result of the time it takes for the compounds to cross the blood-brain barrier and reach therapeutic concentrations within the brain.[52] The progressive anxiolytic effects of BE may be due to its bioactive compounds which resulted in synergistic effects that may modulate neurotransmitter systems and HPA (hypothalamic-pituitary-adrenal) axis and reduce oxidative stress and inflammation. However, the neurotransmitter levels (e.g., serotonin), corticosterone levels in the HPA axis, or markers of oxidative stress and inflammatory cytokines were not evaluated in this current study. Therefore, evaluating these parameters in future studies would help clarify the mechanisms underlying BE's anxiolytic effects and further support its potential as a natural alternative to current pharmacological treatments for anxiety.

Conclusion

In conclusion, this study investigates the anxiolytic effects of BE in a mouse anxiety model, with results suggesting potential efficacy, particularly at the higher dose of 200 mg/kg (BE 200). Specifically, this higher dose increased the time spent in the open arms of the EPM test and the light compartment of the LDB test, which indicates reduced anxiety. However, no significant differences in body weight were observed among the treated groups. Overall, these findings support the potential anxiolytic properties of BE, possibly through neurotransmitter modulation and antioxidant activity, highlighting the need for further investigation into its mechanisms and therapeutic potential.

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F07

Investigation of the Effect of Hypoxic Preconditioning of Clinical-grade Human Adipose-Derived Mesenchymal Stem Cells Exosome (ADSC-Exos) on Carrageenan (CA)-Induced Paw Edema in a Rat Model

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Abstract

Human mesenchymal stem cell (MSC)-derived exosomes have been widely studied to be utilized as cell-free therapies for treating various diseases due to their regenerative, anti-inflammatory, and immunomodulatory properties. The paracrine effects of MSC-derived exosomes are also shown to contribute to these therapeutic benefits. Adipose-derived mesenchymal stem cells (ADSCs) can be simply isolated from adipose tissue, which is much less invasively collected from donors or patients. It has been reported that the contents of exosomes can be modulated by preconditioning cell culture conditions, such as hypoxia and selection of culture medium. In this study, we investigated the effect of ADSC-derived exosome (ADSC-Exos) cultured under hypoxic preconditioning in a clinical-grade, chemically defined formulation medium, compared to normoxic conditions, using a CA-induced rat paw edema model. The characteristics of ADSC-Exos were confirmed prior testing in animal using western blot. Acute paw inflammation was induced by intraplantar (i.p.l.) injection of 100 µL of 1% w/v CA into the right hind paw of male Wistar rats. One hour after CA injection, animals received either i.p.l. treatment with phosphate-buffered saline (PBS) or exosomes (100 µg), or an intraperitoneal injection of indomethacin (10 mg/kg). The anti-inflammatory effects of ADSC-Exos were assessed by pain-related behavioral tests (von Frey filament and weight-bearing tests) at 0, 3, 6, and 24 hours after CA induction. Histological analysis of the inflamed hind paw was also conducted to assess inflammatory cells infiltration. The results showed that both normoxic and hypoxic ADSC-Exos groups exhibited significant pain attenuation; however, the normoxic condition showed significantly greater pain reduction compared to the hypoxic condition. Similarly, histological findings revealed that normoxic ADSC-Exos appeared to exert superior anti-inflammatory effects. These findings are inconsistent with previously published studies, which reported that exosomes derived under hypoxic preconditioning demonstrated greater therapeutic potential than those obtained under normoxic conditions. Further studies are needed to investigate the factors that may be responsible for the inconsistency observed in the present findings.

Keywords: Adipose-derived mesenchymal stem cells (ADSCs), exosomes, anti-inflammation, hypoxic, normoxic, carrageenan (CA)-induced paw edema, pain

Introduction

Mesenchymal stem cells (MSCs), a type of cell-based therapy has been used due to its therapeutic potential for the treatment of several human diseases, including bone and cartilage diseases, neurodegenerative disease, acute brain injury and so on.[1, 2] This due to its ability to regulate the inflammatory and regenerative environment in inflamed tissues.[3] Owing to these unique properties, MSCs hold tremendous promise for treating inflammatory diseases. MSCs can be derived from different tissue sources including bone marrow, adipose tissue, dental tissue, placenta and umbilical cord. Adipose tissue serves as a common MSCs source which are easily and extensively isolated compared to other tissue. Several studies found that the potential of MSCs is mediated through their paracrine secretions including growth factors, chemokine as well as extracellular vesicles (EVs) that may be responsible for intercellular communication, especially exosomes that contain important cargo such as complex protein, nucleic acid, lipid, and miRNA.[4, 5]

Several studies demonstrated the promising results of MSCs-derived exosome treatment in various diseases such as skin injury, spinal cord injury, lung fibrosis, bone and cartilage defects.[6] The properties of exosomes can be varied depending on the tissue source of MSCs, as well as cell culture methods.[7, 8] In general, MSCs are typically cultured under normoxic conditions (21% oxygen). However, in physiological conditions, MSCs thrive in hypoxic environments – e.g. 1-9% in the bone marrow, 5-9% in the adipose tissue and 1-6% in umbilical cord blood.[9-11] It has been suggested that culturing MSCs in hypoxic conditions can mimic physiological environments, which can reduce cellular senescence and alter its therapeutic activity.[12] Recently, several studies demonstrated that hypoxic preconditioning could affect the physiological function of MSCs as well as the release, cargo, and biological effects of exosomes.[13] In spinal cord injury, they found that ADSCs-hypoxia exosomes were more effective than normoxia in terms of faster healing, greater endothelial cord formation and grater vascular density in spinal cord.[14] In a bone fracture healing model, hypoxic exosomes can promote therapeutic potential by enhancing the production of miR-126, which plays an important role in the bone fracture healing process. [15] However, the majority of previous studies on MSCs demonstrated the efficacy and biological activities of cells cultured in medium containing fetal bovine serum (FBS), which is non-compliance for clinical use. FBS contains undefined mixture of proteins and therefore causes batch-to-batch variations and inconsistency quality of exosomes. Moreover, the presence of xenoproteins in FBS that are carried over into harvested exosome fractions may trigger immune response in human recipients. Therefore, in this study, the standardized production procedures of ADSC-Exos under hypoxic preconditioning in clinical-grade chemically defined medium in comparison with normoxic conditions were conducted and their efficacy were determined in a CA-induced rat paw edema model.

Methods

ADSC-Exosome isolation and characterization

Human MSCs from adipose tissue were collected from the Faculty of Medicine, Ramathibodi Hospital, Mahidol University. MSCs from adipose tissue, which is considered left over tissue was obtained according to protocol approved by the human research ethics committee (COA. No. MURA2023/712).

Harvested cells were cultured in NutriStem®XF Basal Medium, 5.5% PLTGold® Human Platelet Lysate and 0.6% MSC Nutri®Stem XF Supplement. Cells are plated into a sterile flask at the density of 0.1-0.5 mcell/cm^2 and cultured at 37 °C, 95% humidity and 5% CO_2 in

normoxia (20% O_2) or hypoxia (3% O_2). Immunophenotypes of human MSCs were defined with flow cytometry by their surface marker profile, including $CD73^+$, $CD105^+$, $CD90^+$, $CD3^-$, $CD34^-$ and $CD45^-$, as well as their differentiation potential to osteocytes and adipocytes were also assessed by Oil Red O staining and Alizarin red staining, respectively.

For ADSC-Exos isolation and characterization, collected cell culture supernatant was centrifuged at 300 g for 10 minutes, and at 16,500 g for 30 minutes. The supernatant was collected and filtered through a 0.22 μm pore size filter to remove cell debris. The remaining supernatant was centrifuged at 100,000 g for 30 minutes and the exosomes were collected in the pellet. Resuspend the purified exosomes with phosphate-buffered saline (PBS) for further characterization. Surface marker proteins of exosomes were identified with western blot using antibodies specific for CD63, TSG101, and CD81 according to International Society for Extracellular Vesicles (ISEV) guidelines. The presence of exosome cargos was also tested with western blot using specific antibodies targeting Transforming growth factor-β (TGF-β) and Matrix metalloproteinase-9 (MMP-9).

CA and drug preparation

The 1% W/V solution λ-CA was prepared in 0.9% saline and stored at room temperature for 24 hours before use. Indomethacin was prepared in 0.01% carboxymethyl cellulose (CMC) containing 0.01% tween 80. It was used as a positive control in this study due to its well-established anti-inflammatory and analgesic properties.

Experimental groups design

All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Science, Mahidol University (Protocol No MUSC67-013-718). The 6-7-week-old male Wistar rats were anesthetized with 5% isoflurane and maintained at 2-3% during CA-induction. Acute inflammation was induced by intraplantar injection with 100 μL of 1% w/v CA at the right hind paw. After 1 hour of CA induction, animals were divided into four different groups as shown below. For the positive control group, animals were intraperitoneally injected with 10 mg/kg of indomethacin, while the remaining groups received an intraplantar injection with either PBS as a control vehicle or human normoxic- or hypoxia-ADSCs exosomes at a dosage of 100 μg/100 μL. One additional group of animals were assigned as a negative control group, in which animals received an intraplantar injection with 100 μL normal saline (NSS), followed by 100 μL PBS after 1 hour post-NSS injection.

Twenty rats were divided into 5 groups:

(1) CA + PBS (n=4)
(2) CA + Indomethacin (10 mg/kg) (n=4)
(3) CA + Normoxic-ADSCs Exos (n=4)
(4) CA + Hypoxic-ADSCs Exos (n=4)
(5) NSS + PBS (n=4)

Pain-related behavior assessment

Two pain-related behavior tests, including von Frey filament test (VF) and weight bearing test (WB) were conducted at various time points to determine the pain-relieving efficacy of the human ADSCs exosomes. The paw withdrawal thresholds (PWT) were measured to assess the mechanical allodynia by using von Frey filament (Touch Test® Sensory Evaluator). In brief, animals were placed individually in wire mesh cages, in which a series of

filaments (2, 4, 6, 8, 10, 12, 14, 16 grams) were applied onto the midplantar area of the right hind paw. The smallest filament that evoked paw withdrawal responses on 3 trials was recorded. The cut-off value for negative response was set at 16 grams to avoid the injury to the animal hind paw. Asymmetric weight distribution (weight bearing test) was also utilized to index musculoskeletal discomfort caused by paw edema. This test measures the weight distributed between the right and left hind paw. Animals were placed into the Perspex container, and each paw was rested on a transducer pad, which recorded as animal's weight distribution. The average percentage of different weight distributed on the operated limb was determined. All animals were investigated in pain-related behaviors at 0, 3, 6, and 24 hours after CA-induction (**Figure 1**).

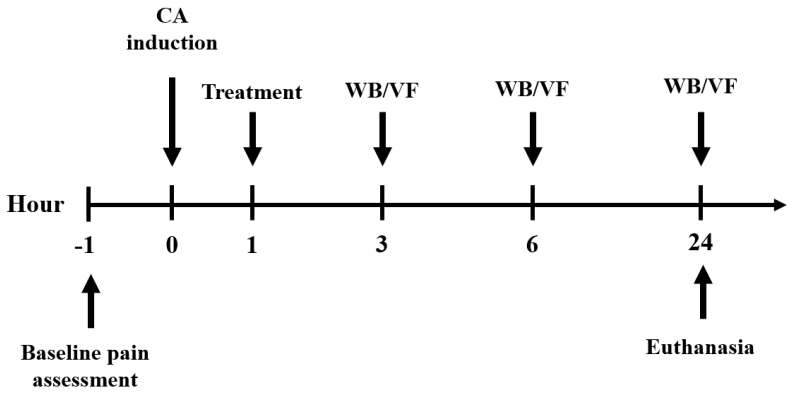

Figure 1. Schematic diagram summarizing the experimental design

Histological examination

After euthanasia with an overdose of thiopental sodium (150 mg/kg) and xylazine (5 mg/kg) via intraperitoneal injection, the right hind paw was dissected and cut in half. Paws were fixed in 4% paraformaldehyde for 72 hours, followed by decalcification in 20% ethylenediaminetetraacetic acid (EDTA) with continuous stirring for at least 2 months. After finishing tissue processing, tissue will be embedded in paraffin. Then the tissue section (5 μm thick) was stained with hematoxylin and eosin (H&E) and visualized under a light microscope.

Statistical analysis

Data were analyzed using the GraphPad Prism version 8.0 (GraphPad Software Inc., San Diego, CA, USA). All data were expressed as the mean and standard error of the mean (MEAN ± SEM). Statistical significance was determined using two-way repeated measures ANOVA follow by post hoc Tukey's multiple comparison. *$p < 0.05$ were considered statistically significant.

Results

Characterization of normoxic- and hypoxic-ADSCs for its identity, purity and potency

The identity of ADSCs cultured under normoxic and hypoxic conditions were characterized according to the extracellular markers specified by the International Society of Cell and Gene therapy (ISCT) that specified >95% expression of CD73, CD105 and CD90. Both normoxic and hypoxic-ADSCs showed higher than 95% expression of MSC markers

(**Figure 2A**) and showed less than 2% of CD3, CD34, and CD45 positive population (**Figure 2B**), which demonstrates that both normoxic and hypoxic cell culture condition produces pure MSC population. The potency of MSCs were evaluated by its ability to differentiate into osteocytes and adipocytes in differentiation medium. Both normoxic and hypoxic cultured ADSCs showed ability to differentiate into osteocytes and adipocytes as shown in **figure 2C**.

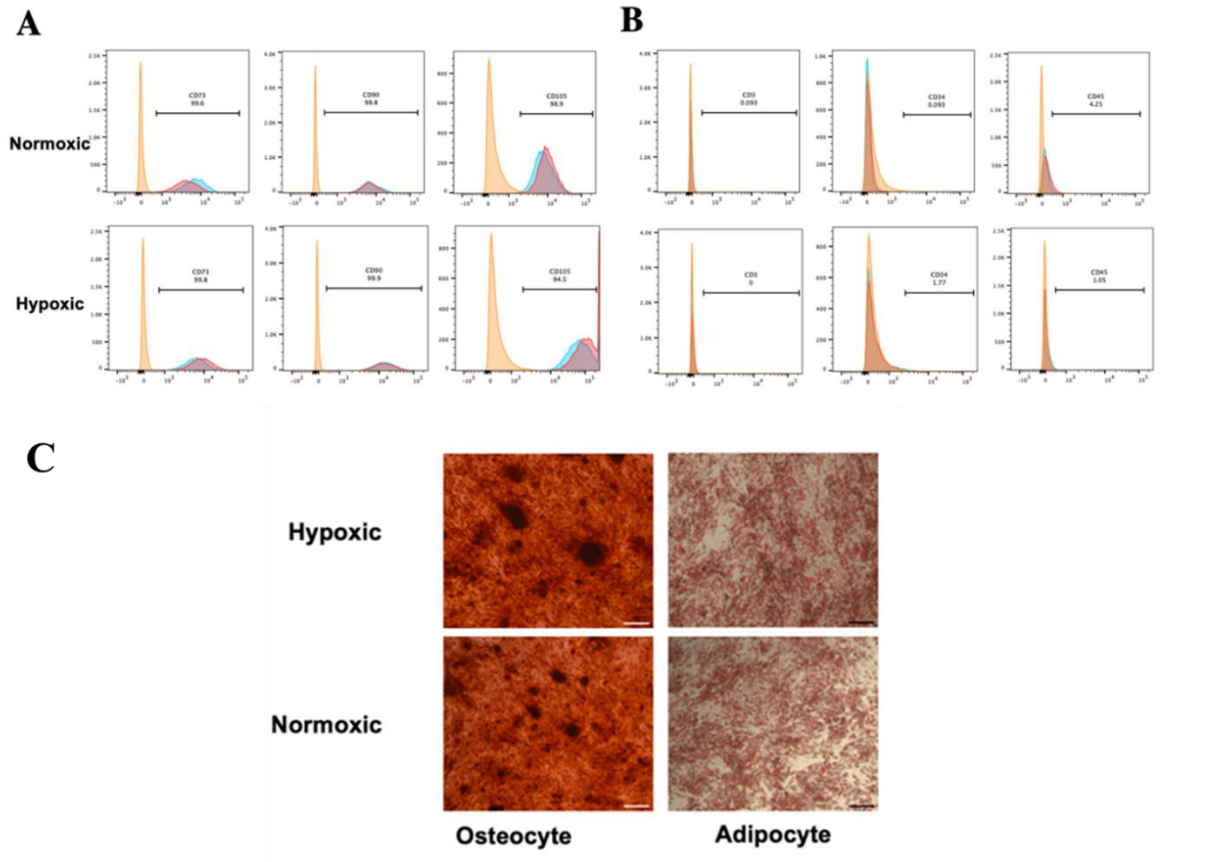

Figure 2. Characterization of ADSCs cultured in normoxic and hypoxic conditions A) Identity of MSCs according to ISCT B) purity of MSC population C) potency of MSCs in differentiating into osteocytes and adipocytes. The scale bar is 60μm.

Characterization of exosomes harvested from ADSCs cultured in normoxic and hypoxic conditions

The protein content of exosomes was characterized to determine the identity of the harvested material. CD63, CD81 and TSG101 are tetraspanins, enriched in exosomes and are commonly used as markers. Exosomes harvested from normoxic and hypoxic cultured ADSCs were positive for CD63, CD81 and TSG101, as well as TGF-β and MMP-9. Interestingly, exosomes harvested from hypoxic-ADSCs showed lower CD63 expression than in normoxic conditions. In addition, MMPs, along with other proteinases and glycanases are known to degrade proteoglycans, collagen and ECM molecules. [16] They were found to be related in the pathogenesis of many diseases, especially joint disorders such as osteoarthritis and rheumatoid arthritis. Exosomes secreted from immune cells (B cells, T cells and dendritic cells) can trigger the upregulation of MMPs, which drives the progression of joint inflammation.[17] Since, it has been reported that MMP-9 is carried in exosomes derived from

MSCs, so we performed western blot analysis to determine the presence of this enzyme.[18] Our western blot data showed that a trace amount of MMP-9 is present in the exosome derived from hypoxic and normoxic conditioned MSCs at comparable amounts. This confirms that neither preconditioning method upregulated MMP-9 cargo in MSC-derived exosomes, which could be unfavorable as a treatment modality for joint inflammation.[19] TGF-β, on the other hand, has been demonstrated to be crucial in cartilage homeostasis and has chondrogenic regenerative potential for repairing cartilage degeneration. Hence, we determined the presence of TGF-β cargo, and found that the cytokine is present in both hypoxic and normoxic conditioned exosomes (**Figure 3**).

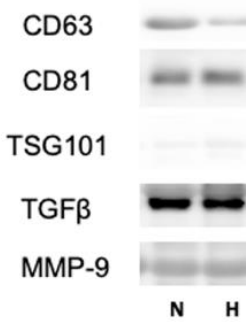

Figure 3. Western blot analysis of exosomal surface proteins markers including CD63, CD81, TSG101, TGF-β and MMP-9 harvested from normoxic (N; left) and hypoxic (H; right) ADSCs.

Normoxic- and hypoxic-ADSCs exosomes reduced CA-induced pain-related behaviors.

The pain relief of normoxic- and hypoxic-ADSCs exosome on CA-evoked mechanical allodynia and weight bearing asymmetry were evaluated by von Frey filament test and weight bearing test respectively. The negative control group (CA + PBS) exhibited a significant reduction in paw withdrawal thresholds (PWT) and %weight on injured hind limb compared with the positive control group of CA+10 mg/kg of indomethacin. Interestingly, treatment with normoxic-ADSCs exosome for 6 hours exhibited a significant pain-reliving effect as indicated by the significant increase in the PWT (**Figure 4A**) and % weight on an injured hind limb (**Figure 4B**) compared to the negative control group (CA+PBS). However, a significant pain alleviation was observed in both pain-like behavioral tests after treatment with both normoxic-and hypoxic-ADSCs exosomes at 24 hours post CA-injection. Surprisingly, this effect was significantly more pronounced after treatment with normoxic-ADSCs exosomes than those with hypoxic-ADSCs exosomes.

Histological findings for the effect of normoxic- and hypoxic-ADSCs exosomes on paw edema tissue induced by CA.

To assess the anti-inflammatory efficacy of normoxic- and hypoxic-ADSCs exosomes, a histopathological examination was investigated based on inflammatory cell infiltration in the plantar area as shown in **Figure 5**. Macroscopic appearance of paw edema in each treatment group was captured and assessed, compared to the control group (NSS + PBS). Visually, anti-inflammatory effect of normoxic-ADSCs exosomes appeared to be greater in comparison to

hypoxic- ADSCs exosomes treated groups when compared to CA+PBS group (**Figure 5A**). Consistent with this observation, microscopic images of soft tissue of the rat hind paw stained with H&E revealed that normoxic-ADSCs exosomes treated group showed a marked anti-inflammatory effect comparable to those positive control group (CA + indomethacin) as observed by a pronounced reduction in the inflammatory cell infiltration in the hind paw tissue (**Figure 5B**).

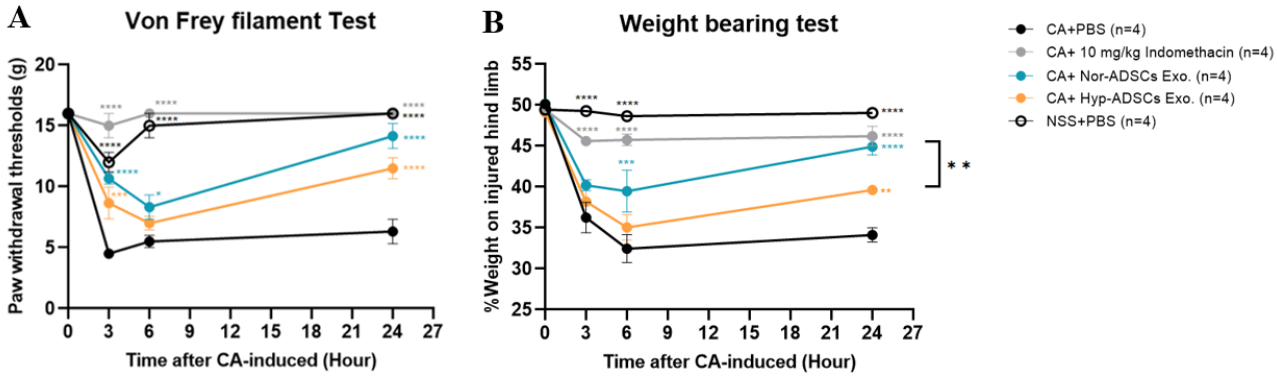

Figure 4. Pain-related behavior assessment of normoxic- and hypoxic-ADSCs Exosome at 0, 3, 6, and 24 hours post-CA induction. (A) von Frey filament test (B) weight bearing test. Data represented as the mean ± SEM in each group and were analyzed using a two-way ANOVA with Tukey's multiple comparisons test. *p < 0.05, ***p < 0.001, ****p < 0.0001 vs CA+PBS group shows statistical significance.

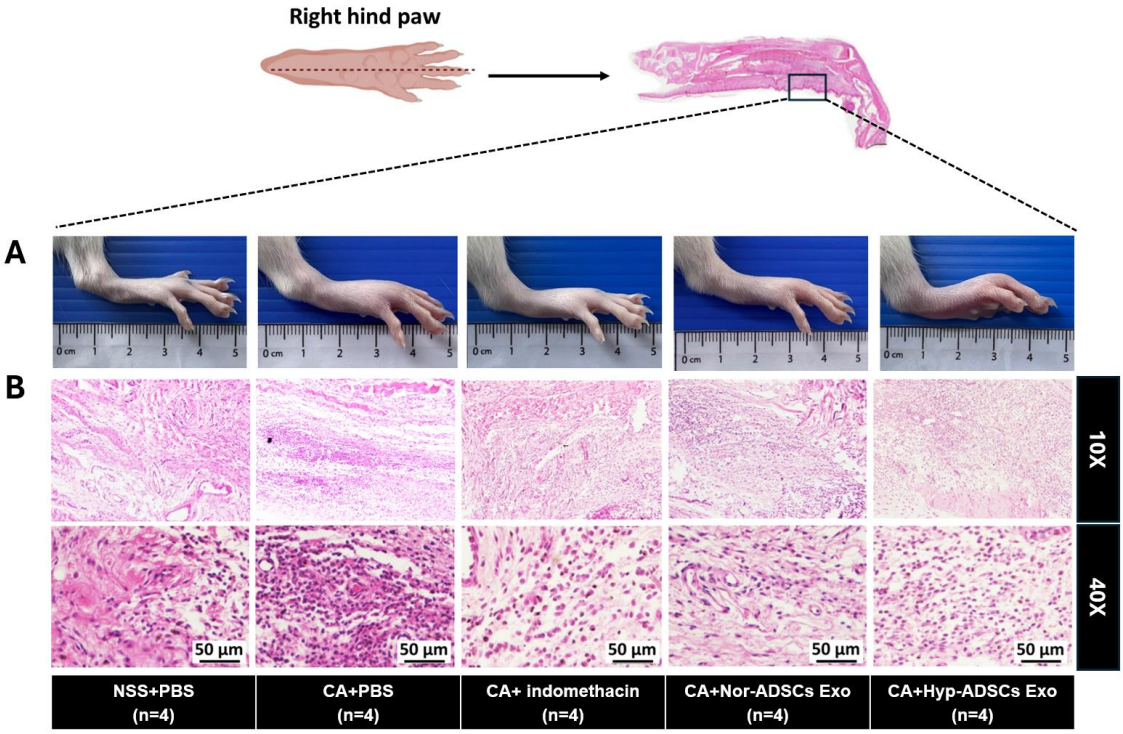

Figure 5. Effect of normoxic- and hypoxic-ADSCs exosomes on paw edema induced by CA in rats. **(A)** Representative photographs of the macroscopic appearance of paw edema in each group (n=4) after treatment at 24 hours after CA-induction. **(B)** Representative histological sections of right hind paw tissue in H&E staining.

Discussion and Conclusion

A growing body of evidence suggests that MSCs-derived exosomes hold great promise for several therapeutic applications, particularly in regenerative medicine. However, much less attention has been paid to standardize the production procedures of MSCs-derived exosomes and investigate whether this procedure could modulate the therapeutic efficacy of exosomes. It has been demonstrated that MSCs-derived exosomes cultured under serum-free chemically defined media exhibited higher expression of regeneration-related cytokines and fewer pro-inflammatory cytokines, compared to serum containing media, which in turn could enhance wound healing and angiogenesis.[20] Our findings demonstrated that the expression of exosome-associated markers (CD63, TSG101, and CD81) and cargo proteins (TGF-β and MMP-9) of ADSC-Exos harvested from normoxic and hypoxic conditions were similar. However, a more quantitative assay to evaluate specific protein content may uncover the variation between normoxic and hypoxic preconditioned exosomes, as their therapeutic efficacy in attenuating inflammation shown in this study is distinct.

Interestingly, while several other findings reported the beneficial effect of hypoxic preconditioning in promoting anti-inflammation, angiogenesis and repair by modulating the miRNA and proteomic profiles[21, 22], we found that exosomes harvested from hypoxic-ADSCs were able to reduce inflammation to less extent than that of exosomes harvested from normoxic conditions. The hypoxic conditions have been shown to upregulate hypoxia-inducible factor (HIF)-alpha, a key regulator of inflammation, which in turn can influence the quantity and expression of exosome cargos.[23] Hypoxic condition has also been reported to cause alteration in sorting and rearrangement of the cytoskeleton and extracellular matrix of exosomes, which influences the reprogramming of recipient cells.[24] Numerous studies indicate that miRNA-containing exosomes can be upregulated under hypoxic conditions.[24-26] Since the biological activities of exosomes are influenced by various factors such as hypoxic and cell culture conditions, it is possible that the differences of these factors among studies could attribute to the conflicting finding observed in this study. However, as this study is preliminary, more research is required to thoroughly comprehend this conflicting finding.

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คำสั่งมหาวิทยาลัยสงขลานครินทร์

ที่ ๐๗๘๑/๒๕๖๘

เรื่อง ยกเลิกและแต่งตั้งคณะกรรมการจัดงานประชุมวิชาการสมาคมเภสัชวิทยาแห่งประเทศไทย ครั้งที่ ๔๖ ประจำปี พ.ศ. ๒๕๖๘ (The 46th Pharmacological and Therapeutic Society of Thailand Annual Meeting 2025) "Innovative and Sustainable Pharmacological Approaches for Combating Non-Communicable Diseases (NCDs)" (ชุดใหม่)

ตามคำสั่งมหาวิทยาลัยสงขลานครินทร์ที่ ๐๖๘๘/๒๕๖๘ ลงวันที่ ๘ พฤษภาคม ๒๕๖๘ ได้แต่งตั้งคณะกรรมการจัดงานประชุมวิชาการสมาคมเภสัชวิทยาแห่งประเทศไทย ครั้งที่ ๔๖ ประจำปี พ.ศ. ๒๕๖๘ (The 46th Pharmacological and Therapeutic Society of Thailand Annual Meeting 2025) " Innovative and Sustainable Pharmacological Approaches for Combating Non-Communicable Diseases (NCDs)" แล้วนั้น

เนื่องด้วยมีการแก้ไขปรับเปลี่ยนเพิ่มเติมรายชื่อคณะกรรมการฯ หลายส่วน เพื่อให้การดำเนินงานเป็นไปด้วยความเรียบร้อยบรรลุวัตถุประสงค์ และมีประสิทธิภาพอาศัยอำนาจตามความในมาตรา ๓๔ แห่งพระราชบัญญัติมหาวิทยาลัยสงขลานครินทร์ พ.ศ. ๒๕๕๙ จึงขอแต่งตั้งคณะกรรมการจัดงานประชุมวิชาการระดับชาติสรีรวิทยาสมาคมแห่งประเทศไทย ครั้งที่ ๕๒ ประจำปี พ.ศ. ๒๕๖๘ ชุดใหม่ โดยมีรายนามดังต่อไปนี้

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๑. ให้คำปรึกษาและแนวทางในการดำเนินงานให้แก่คณะกรรมการฝ่ายต่าง ๆ เพื่อให้การจัดประชุมวิชาการครั้งนี้ดำเนินไปตามวัตถุประสงค์

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๒๔.	ผู้ช่วยศาสตราจารย์ ดร.ภญ.บัณฑิตาภรณ์ ศิริจันทร์ชื่น	กรรมการ
๒๕.	รองศาสตราจารย์ ดร.น.สพ.รักธรรม เมฆไตรรัตน์	กรรมการ
๒๖.	ผู้ช่วยศาสตราจารย์ ดร.วันทณี หาญช้าง	กรรมการ
๒๗.	ผู้ช่วยศาสตราจารย์ ดร.บงกช ธุระธรรม	กรรมการ

/๒๘.ผู้ช่วยศาสตราจารย์ ดร.มรกต...

๒๘. ผู้ช่วยศาสตราจารย์ ดร.มรกต สร้อยระย้า กรรมการ
๒๙. ผู้ช่วยศาสตราจารย์ ดร.เพ็ญนภา ชลปฐมพิกุลเลิศ กรรมการ

<u>ฝ่ายตัดสินการนำเสนอผลงาน</u>

๓๐. ผู้ช่วยศาสตราจารย์ ดร.ภัควดี เสริมสรรพสุข กรรมการ
๓๑. รองศาสตราจารย์ ดร.ภญ.นนทญา นาคคำ กรรมการ
๓๒. รองศาสตราจารย์ ดร.ภญ.ศริญญา คงเพชร กรรมการ
๓๓. ผู้ช่วยศาสตราจารย์ ดร.เตือนใจ ขุนรักษ์ กรรมการ
๓๔. ผู้ช่วยศาสตราจารย์ ดร.สมชาย ศรีวิริยะจันทร์ กรรมการ
๓๕. ดร.ภก.วริทธิ์ เรืองเลิศบุญ กรรมการ
๓๖. ศาสตราจารย์ ภก.ดร.ปิติ จันทร์วรโชติ กรรมการ
๓๗. รองศาสตราจารย์ ดร.ภญ.มยุรี ตันติสิระ กรรมการ

มีหน้าที่ ดังนี้

๑. พิจารณา กำหนดรายละเอียดเนื้อหา หัวข้อประชุม และคัดเลือกบทความวิจัยที่จะนำเสนองานแบบ Poster Presentation
๒. จัดทำ Template Abstract / Template Proceeding
๓. กำหนดรูปแบบในการจัดทำบทคัดย่อ
๔. กำหนดรูปแบบในการจัดทำโปสเตอร์
๕. พิจารณาคัดเลือกและประสานงานกับผู้ทรงคุณวุฒิเบื้องต้นเพื่อพิจารณาบทความวิจัย
๖. กำหนดระยะเวลาการประเมินบทความวิจัยให้แก่ผู้ทรงคุณวุฒิ
๗. จัดส่งบทความผ่านระบบเพื่อให้ผู้ทรงคุณวุฒิพิจารณาบทความ
๘. จัดทำกำหนดการการนำเสนอผลงาน
๙. รวบรวมบทความและจัดทำ Abstract Book / Proceeding
๑๐. พิจารณาและดำเนินการในส่วนที่เกี่ยวข้องกับวิชาการเพื่อให้งานประชุมเรียบร้อยและมีประสิทธิภาพ
๑๑. ควบคุมและกำกับเวลาในการนำเสนอผลงาน
๑๒. ประสานงานฝ่ายวิชาการ
๑๓. พิจารณาคัดเลือกคณะกรรมการประจำ session
๑๔. กำหนดเกณฑ์การประเมินการนำเสนองานแบบ poster presentation
๑๕. กำหนดรางวัลการนำเสนอผลงาน

คณะกรรมการระบบลงทะเบียน ลงทะเบียน เกียรติบัตรออนไลน์ และของที่ระลึก

๑. ผู้ช่วยศาสตราจารย์ ดร.ภญ.สุพัตรา ลิ่มสุวรรณโชติ ประธานกรรมการฝ่าย
๒. ดร.กรณ์สุดา ทิพย์อาสน์ รองประธานฝ่าย
๓. ผู้ช่วยศาสตราจารย์ ดร.สถาพร พฤฒิพรรลาย กรรมการ
๔. คุณปรีดารัตน์ ประไพ กรรมการ
๕. ดร.ภญ.วรวีร์ ศิริพฤกษ์พงศ์ กรรมการ
๖. นางสาวกนกวรรณ สุภาคำ กรรมการและเลขานุการ
๗. นางสาวนงคราญ เอ่งฉ้วน กรรมการและผู้ช่วยเลขานุการ

/ฝ่ายระบบ...

ฝ่ายระบบลงทะเบียน และเกียรติบัตรออนไลน์

๘.	นางอรอุมา รัตนดิลก ณ ภูเก็ต	กรรมการ
๙.	นายภูษิต แซ่อุย	กรรมการ
๑๐.	นายวัธกร เจริญสิริสกุล	กรรมการ
๑๑.	นายสถาพร สุวรรณ	กรรมการ
๑๒.	นางสาวอัญชลี พัฒนพันธ์ชัย	กรรมการ
๑๓.	นางสาวอรุณศรี รัตนญา	กรรมการ
๑๔.	นายบัญชา ขำพิทักษ์	กรรมการ
๑๕.	นายชัยวัฒน์ พานิชกุล	กรรมการ

ฝ่ายรับลงทะเบียน และ ของที่ระลึก

๑๖.	นางสาวจิระภา พันธรัตน์	กรรมการ
๑๗.	นางสาวภัทรานิษฐ์ ชูบัวทอง	กรรมการ

มีหน้าที่ ดังนี้

๑. พัฒนาระบบเพื่อใช้สำหรับการลงทะเบียนและจัดส่งบทความออนไลน์
๒. สามารถตอบคำถามและแก้ปัญหาระบบการลงทะเบียนให้แก่ผู้ใช้ระบบได้
๓. ดูแลความเรียบร้อยของระบบลงทะเบียนและระบบการจัดส่งบทความออนไลน์
๔. จัดทำระบบเกียรติบัตรออนไลน์
๕. จัดทำข้อมูลและสรุปรายชื่อผู้ลงทะเบียนและผู้เข้าร่วมประชุมทั้งหมด (ผู้เข้าร่วมงานทุกประเภท)
๖. ตรวจสอบรายชื่อเพื่อจัดพิมพ์ป้ายชื่อและเกียรติบัตร (ส่งรายชื่อให้ฝ่ายประชาสัมพันธ์ฯ)
๗. จัดเตรียมป้ายชื่อและของที่ระลึก ให้แก่ผู้ลงทะเบียนทุกท่าน
๘. กำหนดรูปแบบการรับลงทะเบียนหน้างาน
๙. ดำเนินการให้การลงทะเบียนเป็นไปอย่างเรียบร้อย
๑๐. จัดหา / ส่งของที่ระลึกให้แก่สปอนเซอร์
๑๑. จัดหาของที่ระลึกให้แก่ทีมวิทยากรและผู้เข้าร่วมงาน
๑๒. จัดส่งของที่ระลึกให้แก่วิทยากร กรณีอยู่ต่างประเทศ (ถ้ามี)

คณะกรรมการฝ่ายโสตทัศนูปกรณ์ เทคโนโลยี ประชาสัมพันธ์ เว็บไซต์ และจัดทำสื่อ

๑.	ดร.ภก.วริทธิ์ เรืองเลิศบุญ	ประธานกรรมการฝ่าย
๒.	ผู้ช่วยศาสตราจารย์ ดร.วันดี อุดมอักษร	กรรมการ
๓.	รองศาสตราจารย์ ดร.วนิดา สุขเกษศิริ	กรรมการ
๔.	นางสาวธนภัทร สุระกุล	กรรมการและเลขานุการ
๕.	นายภานุมาศ อ่อนมาก	กรรมการและผู้ช่วยเลขานุการ

ฝ่ายโสตทัศนูปกรณ์ เทคโนโลยี

๖.	นางอรอุมา รัตนดิลก ณ ภูเก็ต	กรรมการ
๗.	นายธนาวัฒน์ เที่ยงตรง	กรรมการ
๘.	นายธนนชัย เรืองรักษ์	กรรมการ
๙.	นายวัชรพงศ์ ศรีทา	กรรมการ

/ฝ่ายประชาสัมพันธ์...

ฝ่ายประชาสัมพันธ์ เว็บไซต์ และจัดทำสื่อ

๑๐. นายอิสรภาพ ชุมรักษา กรรมการ
๑๑. นายวณิกบุตร วัฒนมติพจน์ กรรมการ
๑๒. นางสาวชัญญา อินทกาญจน์ กรรมการ
๑๓. นายไซฟู สะมะโด กรรมการ

มีหน้าที่ ดังนี้

๑. ควบคุมระบบแสง สี เสียง ในพิธีเปิด-ปิด และงานเลี้ยงในงานประชุม
๒. จัดเตรียมและจัดหาอุปกรณ์โสตทัศนูปกรณ์ต่าง ๆ ให้ทำงานได้และมีประสิทธิภาพ
๓. จัดเตรียมอุปกรณ์โสตทัศนูปกรณ์สำหรับการนำเสนอแบบ Oral Pressentation
๔. ตรวจสอบการใช้งานอุปกรณ์โสตทัศนูปกรณ์ต่าง ๆ เมื่องานประชุมเสร็จสิ้นในแต่ละวัน
๕. ประสานงานกับฝ่ายอุปกรณ์ของทางโรงแรม
๖. จัดทำโลโก้ เว็บไซต์ และคลิปวิดีโอเพื่อประชาสัมพันธ์งานประชุมวิชาการ
๗. จัดทำโปสเตอร์ประชาสัมพันธ์งานประชุมวิชาการ
๘. ดูแล และปรับปรุงข้อมูลบนเว็บไซต์การประชุมให้ทันสมัยอยู่เสมอ
๙. ประสานงานกับฝ่ายต่าง ๆ ในการแขวนข้อมูลบนเว็บไซต์
๑๐. ออกแบบ และ จัดทำ Artwork ที่เกี่ยวข้องกับงานประชุมวิชาการทั้งหมด
๑๑. จัดเตรียมวิดีโอสำหรับงานประชุมวิชาการในพิธีเปิด
๑๒. ประชาสัมพันธ์งานประชุมวิชาการผ่านช่องทางต่าง ๆ
๑๓. ทำหน้าที่ออกแบบและอื่น ๆ ที่เกี่ยวข้อง

คณะกรรมการฝ่ายที่พัก สถานที่ และ ยานพาหนะ

๑. ผู้ช่วยศาสตราจารย์ ดร.สถาพร พฤฒิพรรลาย ประธานกรรมการฝ่าย

คณะกรรมการฝ่ายที่พัก สถานที่

๒. นายสุทัศน์ ยอดเมฆ กรรมการ
๓. นายนิพนธ์ สุบรรน้อย กรรมการ
๔. นายวัชระธนินทร์ บุญสนิท กรรมการ
๕. นายเจริญจิตต์ ลูกทอง กรรมการ
๖. นางสาวปิยะเนตร ไชยกูล กรรมการ
๗. นางสาวณัฐวสา วรกิจจาภรณ์ กรรมการและเลขานุการ
๘. นางสาวธนภัทร สุระกุล กรรมการและผู้ช่วยเลขานุการ

คณะกรรมการฝ่ายยานพาหนะ

๙. นายเจริญพงศ์ ทิ้งแก้วขาว กรรมการ
๑๐. นายฉัตรชัย บินโสๆ กรรมการ
๑๑. นายปราโมทย์ ย่องตีบ กรรมการ
๑๒. นายมนูญ สิกะพันธ์ กรรมการ
๑๓. นายวัฒนา ชายทองแก้ว กรรมการ
๑๔. นายสมปอง ผจญจิตต์ กรรมการ
๑๕. นางสาวสิริพรรณ จันทวาท กรรมการและเลขานุการ

/๑๖.นางสาวณัฐวสา...

๑๖. นางสาวณัฐวสา วรกิจจาภรณ์ กรรมการและ
ผู้ช่วยเลขานุการ

มีหน้าที่ ดังนี้

๑. จัดเตรียม – เก็บ สถานที่งานประชุมวิชาการตามแบบที่กำหนด
๒. วางแผน ควบคุม การประสานงานด้านการจัดเตรียมความพร้อมด้านสถานที่จัดการประชุม ห้องจัดเลี้ยงสำหรับผู้เข้าร่วมประชุม ห้องจัดเลี้ยงสำหรับคณะกรรมการ ตามความเหมาะสม
๓. วางแผน ควบคุม ตลอดจนการประสานงานด้านการจัดเตรียมด้านอาหารและเครื่องดื่ม ตลอดการจัดประชุม งานเลี้ยงรับรอง สำหรับผู้เข้าร่วมการประชุม
๔. จัดเตรียมอาหารว่าง เครื่องดื่ม ประจำห้องสำหรับผู้เข้าร่วมงานและคณะกรรมการ
๕. จัดทำรายการอาหารและงบประมาณ
๖. วางแผน ควบคุม กำกับ การจัดเตรียมยานพาหนะเพื่อรับส่งผู้เข้าร่วมประชุม ระหว่างที่พักถึงที่ประชุม สนามบินถึงที่ประชุม ตลอดจนความพร้อมด้านยานพาหนะตลอดช่วงการจัดประชุมวิชาการ
๗. แก้ปัญหาเฉพาะหน้าได้
๘. เตรียมบอร์ดสำหรับ poster presentation

คณะกรรมการฝ่ายการเงิน จัดหารายได้ และพัสดุ

๑. ผู้ช่วยศาสตราจารย์ ดร.ภญ.สุพัตรา ลิ่มสุวรรณโชติ ประธานฝ่ายการเงิน
๒. ดร.กรณ์สุดา ทิพย์อาสน์ ประธานฝ่ายจัดหารายได้
๓. นางนาฏยา กัณหาเจริญพงศ์ รองประธานฝ่าย
การเงิน
๔. นางสาวเสาวลักษณ์ รัตนะ รองประธานฝ่ายพัสดุ

<u>ฝ่ายการเงิน</u>

๕. นางสาวบุญไสว ชุ้นสุวรรณ กรรมการ
๖. นางสาวศิริพร ยังสุข กรรมการ
๗. นางสาวอุมาภรณ์ สงขำ กรรมการ
๘. นางสาวจีราณี หมะประสิทธิ์ กรรมการและ
เลขานุการฝ่าย

<u>ฝ่ายจัดหารายได้</u>

๙. ผู้ช่วยศาสตราจารย์ ดร.สถาพร พฤฒิพรรลาย กรรมการ
๑๐. รองศาสตราจารย์ ดร.วนิดา สุขเกษศิริ กรรมการ
๑๑. ผู้ช่วยศาสตราจารย์ ดร.วันดี อุดมอักษร กรรมการ
๑๒. ผู้ช่วยศาสตราจารย์ ดร.สมชาย ศรีวิริยะจันทร์ กรรมการ
๑๓. ผู้ช่วยศาสตราจารย์ ดร.สุชารัศมิ์ ตั้งสุขฤทัย กรรมการ
๑๔. ดร.ภก.วริทธิ์ เรืองเลิศบุญ กรรมการ
๑๕. นางสาวจิระภา พันธรัตน์ กรรมการ
๑๖. นางสาวภัทรานิษฐ์ ชูบัวทอง กรรมการ
๑๗. นางสาวจุรีรัตน์ พรหมจิตร กรรมการและ
เลขานุการฝ่าย

/ฝ่ายพัสดุ...

ฝ่ายพัสดุ

๑๘. นางสาวกรรยา พิทักษ์ฉนวน	กรรมการ
๑๙. นายวีระยุทธ โหล๊ะเตย	กรรมการ
๒๐. นางวิกุล จิตร์นิยม	กรรมการและเลขานุการฝ่าย

มีหน้าที่ ดังนี้

๑. ควบคุม กำกับ และตรวจสอบรายรับ – รายจ่าย ในการจัดงานประชุมวิชาการ
๒. จัดเตรียมใบเสร็จรับเงินให้แก่ผู้เข้าร่วมประชุมทุกท่าน
๓. ขออนุมัติยืมเงินทดรองจ่ายและจัดทำเอกสารประกอบการเบิกจ่ายที่เกี่ยวข้องกับงานประชุมวิชาการ
๔. จัดเตรียมเอกสารประกอบการเบิกจ่ายสำหรับงานประชุมวิชาการ
๕. สรุปรายรับ - รายจ่าย หลังจากงานประชุมวิชาการเสร็จ
๖. จัดทำหนังสือขออนุเคราะห์ / ขอขอบคุณ ในการเป็นสปอนเซอร์ให้กับงานประชุมวิชาการ
๗. ติดตามสปอนเซอร์ในการให้การสนับสนุน
๘. ประสานงานกับฝ่ายการเงินและบัญชี
๙. จัดทำสรุปรายงานการเงินจากสปอนเซอร์
๑๐. จัดเตรียมเอกสารประกอบการจัดซื้อจัดจ้าง พัสดุ-ครุภัณฑ์
๑๑. จัดเตรียมเอกสารหลักฐานต่าง ๆ ที่เกี่ยวข้องทางด้านการเงินและพัสดุ ให้เป็นไปตามหลักเกณฑ์และระเบียบที่กำหนด
๑๒. ควบคุม กำกับ จัดซื้อจัดจ้างวัสดุ อุปกรณ์ประกอบการจัดประชุม การเตรียมเอกสารเพื่อเบิกจ่ายตามหลักฐานการเบิกจ่ายทั้งระหว่างดำเนินงานภายหลังเสร็จสิ้นการดำเนินงาน
๑๓. ประสานงานกับฝ่ายการเงินเพื่อเบิกจ่ายเงิน

คณะกรรมการฝ่าย Low Carbon Event

๑. ผู้ช่วยศาสตราจารย์ ดร.พรสุดา บ่มไล่	ประธานกรรมการฝ่าย
๒. นางสาวนุชรี อินทร์ศรีพราหมณ์	กรรมการ
๓. นางสาวชนากานต์ สงวนศักดิ์	กรรมการ
๔. นางวาวสิริพรรณ จันทวาท	กรรมการ
๕. นางสาววัญญารัตน์ คงสรรพ	กรรมการและเลขานุการ

มีหน้าที่ ดังนี้

๑. ดำเนินการจัดเก็บข้อมูลและแสดงข้อมูลการจัดงานแบบ Low Carbon Event

คณะกรรมการฝ่ายพิธีการ ประสานงาน อาหาร และนันทนาการ

๑. ผู้ช่วยศาสตราจารย์ ดร.สุชารัศมิ์ ตั้งสุขฤทัย	ประธานกรรมการฝ่าย
๒. ดร.กรณ์สุดา ทิพย์อาสน์	รองประธานกรรมการ
๓. ดร.ธนาภรณ์ เฮงประถม	กรรมการ
๔. ดร.บุญญกร บุญศรี	กรรมการ
๕. ดร.ศุภพงศ์ อิ่มสรรพางค์	กรรมการ

/๖.ดร.น.สพ.วิชญะ...

๖.	ดร.น.สพ.วิชญะ ทองตะโก	กรรมการ
๗.	ดร.อมรรัตน์ ชูเกลี้ยง	กรรมการ
๘.	นางสาวจิรัฏฐา ศิริวรรณ	กรรมการ
๙.	นายภานุมาศ อ่อนมาก	กรรมการ
๑๐.	นางสาวกนกวรรณ สุภาคำ	กรรมการ
๑๑.	นางสาวณัฐวสา วรกิจจาภรณ์	กรรมการ
๑๒.	นางสาวธัญญาภรณ์ บุญเพ็ชร	กรรมการ
๑๓.	นางสาวสราญรัตน์ พิทักษ์ปฐมพงศ์	กรรมการ
๑๔.	นางสาวนงคราญ เอ่งฉ้วน	กรรมการ.
๑๕.	นางสาวสุนิสา มีเสน	กรรมการและเลขานุการ
๑๖.	นางสาวธนภัทร ฐระกุล	กรรมการและผู้ช่วยเลขานุการ

มีหน้าที่ ดังนี้

๑. ประสานงานกับตัวแทนที่มีหน้าที่รับผิดชอบทางด้านการประชุมวิชาการกับคณะ/มหาวิทยาลัย รวมถึงเครือข่ายความร่วมมือต่าง ๆ โดยส่งหนังสือ และ E-mail เพื่อเชิญชวนนักวิชาการ นักศึกษาในสาขาที่เกี่ยวข้องให้เข้าร่วมประชุมฯ
๒. จัดทำหนังสือเชิญอธิการบดี รองอธิการบดี และวิทยากร และประชาสัมพันธ์ไปยังหน่วยงานต่างๆ ที่เกี่ยวข้อง
๓. จัดทำกำหนดการพิธีเปิด – ปิดงานประชุม
๔. เตรียมคำกล่าวรายงาน คำกล่าวเปิด คำกล่าวปิด ฯลฯ
๕. ควบคุม และดำเนินการจัดพิธีเปิด – ปิดงานประชุม
๖. รับรองแขก VIP อธิการบดี รองอธิการบดี และวิทยากร
๗. จัดทำรายงานการประชุม สรุปประชุม (เฉพาะการประชุมรวมทุกฝ่าย) และติดตามงานจากฝ่ายต่าง ๆ
๘. จัดทำคำสั่งแต่งตั้งคณะกรรมการจัดการประชุมวิชาการ
๙. จัดทำโครงการพร้อมขออนุมัติโครงการและจัดทำรายงานสรุปปิดโครงการ
๑๐. ประสานงานกับฝ่ายต่าง ๆ รวบรวมข้อมูล และนำส่งฝ่ายที่เกี่ยวข้อง เพื่อการดำเนินงานได้อย่างสะดวก
๑๑. ประสานงานด้านการเตรียมการต้อนรับ ประสานข้อมูล การเดินทางของคณะผู้บริหาร คณะวิทยากรและผู้เข้าร่วมประชุม
๑๒. กำหนดรูปแบบภาพรวมในพิธีการด้านต่างๆ ทั้งก่อนการประชุม ระหว่างการประชุม และหลังเสร็จสิ้นการประชุมกำหนดผู้รับผิดชอบให้การดำเนินงานจัดประชุมเป็นไปด้วยความเรียบร้อยและมีประสิทธิภาพ
๑๓. จัดทำหนังสือเชิญวิทยากรและอาจารย์อาวุโสในช่วงมุทิตาจิต
๑๔. จัดหาพวงมาลัยและดอกกุหลาบให้แก่อาจารย์อาวุโสในช่วงมุทิตาจิต
๑๕. จัดกิจกรรมนันทนาการในช่วง Welcome Party
๑๖. จัดทำแบบประเมิน และประเมินผลการจัดงานประชุมวิชาการ
๑๗. หน้าที่อื่น ๆ ที่เกี่ยวข้องหรือที่ได้รับมอบหมาย

/ทั้งนี้...

ทั้งนี้ ตั้งแต่วันที่ ๑ ตุลาคม ๒๕๖๗ ถึง ๓๑ กรกฎาคม ๒๕๖๘

สั่ง ณ วันที่ ๒๖ พฤษภาคม พ.ศ. ๒๕๖๘

Digitally signed: 2025.05.27 9:31:21 +07:00

(ผู้ช่วยศาสตราจารย์ ดร.นิวัติ แก้วประดับ)
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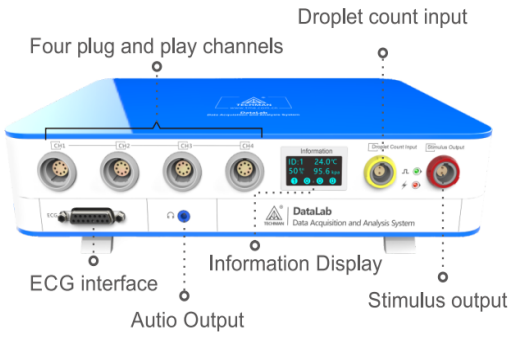

Animal Physiology Lab

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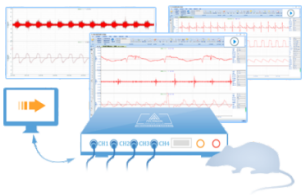

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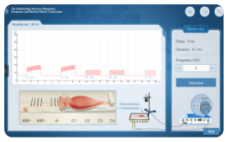
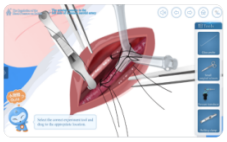
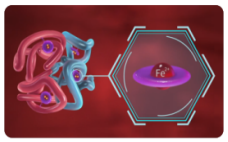

Simulated Interaction


Operation Process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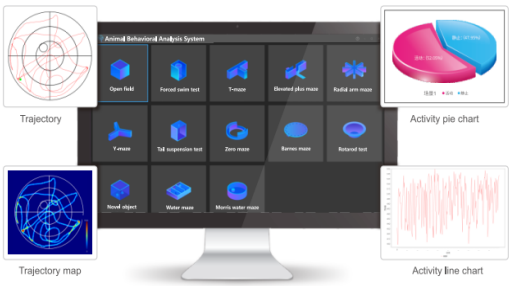

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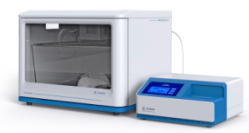
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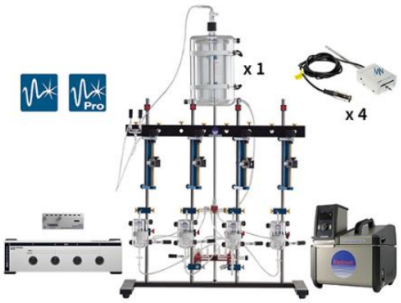

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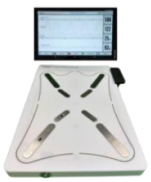

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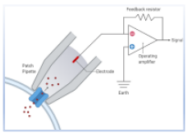

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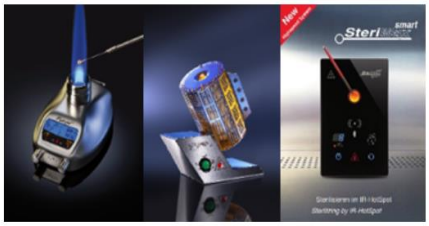

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