Unlocking the potential of luteolin: A natural migraine management approach through network pharmacology

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A B S T R A C T

Background: Luteolin, a natural flavonoid, exhibits antioxidant and anti-inflammatory properties and has been investigated for potential health benefits. Its focus on migraine management arises from its ability to mitigate neuroinflammation, a key factor in migraine attacks.

Methods: pkCSM and Swiss ADME were employed to assess luteolin's pharmacokinetic properties, revealing challenges such as low water solubility and limited skin permeability. OSIRIS Property Explorer is used to check the toxicity. Ligand binding simulations indicated luteolin's potential to interact with calcitonin gene related peptide proteins, crucial in migraine pathophysiology. DisGeNet identified common targets related to migraine, with subsequent network analysis emphasizing promising targets.

Results and Discussion: Luteolin demonstrated good intestinal absorption but faced BBB limitations, suggesting a potential for oral administration but questioning direct brain impact. Nanoformulation was proposed to address solubility challenges, emphasizing the need for in vivo validation. The highest binding affinity with CGRP proteins PDBID: 6PFO (−7.63 kcal/mol) suggested a potential for migraine treatment, requiring empirical confirmation. Enrichment network analysis illustrated luteolin's potential in migraine treatment, emphasizing key targets such as PTGS2, AKT1, ESR1, MMP2, and MMP9. Luteolin shows promise for migraine management, evident in its pharmacokinetic, toxicological profiles, and interactions with CGRP proteins. Challenges like low solubility suggest the need for nanoformulations and empirical validation. Target identification and network analysis offer insights, highlighting potential therapeutic avenues in migraine treatment.

Conclusion: Luteolin holds promise in migraine management, necessitating further research for translation into effective interventions, considering its neuroprotective potential in broader neurological conditions.

1. Background

Migraine arises from complex interactions and pathological functioning of brain circuits influenced by genetic predisposition and various life factors. The expression of migraine is dynamic and influenced by the management of these factors. Shared features with psychiatric comorbidities and behavioral traits contribute to dysfunctional pain processing, forming a self-perpetuating cycle. Environmental factors, such as stigma and lack of support, can worsen the condition. Studying the neuroinflammation milieu and connectivity changes may reveal individualized targets for precision medicine. While the Biopsychosocial (BPS) model holds potential for migraine, its application is intricate, requiring a comprehensive assessment of individuals and their environments.[1] The Global Burden of Disease (GBD) report highlights the continued high prevalence of headache disorders globally and underscores certain methodological factors as contributors to significant variations among study outcomes. These variations introduce uncertainty regarding the trends in migraine prevalence over time and geographical disparities. To address these uncertainties, there is a pressing need for additional and improved studies, particularly in low- and middle-income countries.[2] Sustainable Development Goals (SDG), focusing on ensuring healthy lives and well-being, encompasses vital targets for health improvement. The 2030 Agenda to ensure healthy lives by targeting chronic headaches; curbing the overuse of pain-relieving medications; enhancing healthcare professional education; and facilitating medication access in low- and middle-income countries (LMIC). The analysis emphasizes the impact on health and productivity, particularly for individuals under 50, especially women.[3]

The neuroinflammation hypothesis for migraines proposes that inflammatory processes in the central nervous system (CNS) play a vital role in triggering and sustaining migraine attacks. Increased release of pro-inflammatory molecules, like cytokines, during attacks activates the immune system in the CNS, leading to neuroinflammation. Glial cells, essential for the brain's immune system, can be activated by various triggers, contributing to the release of inflammatory mediators and neuronal sensitization. Dysfunction of the blood-brain barrier allows immune cells and inflammatory molecules into the brain, contributing to neuroinflammation. Cortical Spreading Depression (CSD), linked to the aura phase of migraines, can trigger the release of inflammatory molecules and activate immune responses. Inflammatory processes outside the CNS, in the trigeminal system and blood vessels, may also contribute to central neuroinflammation. The bidirectional communication between the periphery and the CNS is believed to play a role in migraine pathophysiology. Genetic susceptibility and environmental factors influence an individual's predisposition to neuroinflammation in migraines, emphasizing the need to understand the interplay between genetic and environmental triggers.[4]

CGRP or calcitonin gene related peptide is a neuropeptide that plays a significant role in migraine headaches.[5–7] Research into CGRP and its connection to migraines has led to the development of targeted therapies for migraine prevention and treatment. It is a neurotransmitter that is involved in the transmission of pain signals in the nervous system. In people who experience migraines, there is evidence to suggest that CGRP levels are elevated, particularly during a migraine attack.[8] This suggests that CGRP may play a crucial role in the initiation and progression of migraines. It is known to cause vasodilation, believed that the release of CGRP leads to the dilation of blood vessels in the brain. This dilation can be associated with the throbbing pain that many migraine sufferers experience. Researchers have developed medications designed to target CGRP. These include CGRP receptor antagonists such as Erenumab,[9–11] Galcanezumab,[12] Fremanuzumab,[13–15] Eptinezumab,[16–18] Zavegepant,[19–21] and Rimegepant.[22–24] These medications work by blocking the effects of CGRP or reducing the levels of CGRP in the body. In 2022, The European Headache Federation reported that the Monoclonal antibodies that target the CGRP pathway seem to demonstrate effectiveness and safety for preventing migraines, even over an extended period.[25] They are used for both acute treatment of migraines and preventive therapy. CGRP-based therapies have shown promise in clinical trials, with many migraine sufferers reporting significant relief. However, as with any medication, individual responses can vary, and side effects can occur such as constipation, fatigue, and severe cardiac effects.

Luteolin is a flavonoid, a class of naturally occurring compounds found in plants that have been recognized for their potential health benefits. It is a yellow crystalline compound with a bitter taste and is classified as a polyphenol.[26,27] Luteolin is commonly found in various foods and herbs and has been the subject of scientific research due to its potential medicinal properties. Luteolin can be found in a variety of fruits, vegetables, and herbs.[28] Some of the richest dietary sources of luteolin include celery, parsley, thyme, peppers, and carrots. It is also present in foods like artichokes, spinach, and green peppers. Additionally, some teas and nuts contain luteolin.[29] Some research suggests that luteolin may have antioxidant,[30–33] anti-inflammatory,[33] neuroprotective,[34] anticancer,[35] anti-allergic[36,37] properties; due to its antioxidant properties may also be beneficial for the skin. It can help protect the skin from the damaging effects of UV radiation and promote skin health.[38–40] It has been investigated for its potential to support cardiovascular health.[41,42] Luteolin, recognized for potential heart disease risk reduction through improved blood vessel function, reduced oxidative stress, and inflammation inhibition, is gaining attention for its role in mitigating neuroinflammation. Neuroinflammation is implicated in conditions like migraines, and Luteolin's anti-inflammatory and antioxidant properties suggest a potential role in managing neuroinflammation-related migraines. This may lead to a reduction in migraine intensity and frequency by neutralizing harmful free radicals and protecting neurons from inflammation-induced damage. Ongoing research explores Luteolin's neuroprotective benefits in the context of migraines. Luteolin, by reducing neuroinflammation and modulating signaling pathways linked to pro-inflammatory cytokines, may alleviate the pain and discomfort associated with migraines. Targeting these pathways could help regulate neuroinflammatory responses, providing potential relief for migraine sufferers. Migraine attacks often triggered or exacerbated by factors like specific foods, stress, and hormonal changes, can benefit from the potential therapeutic effects of luteolin.[43,44] Incorporating luteolin-rich foods, such as celery and parsley, into a migraine-friendly diet may lower the risk of triggering attacks. However, it's crucial to recognize that while luteolin holds potential for migraine management, individual responses may differ. Migraine is a multifaceted neurological disorder with various triggers and mechanisms, and luteolin or any specific dietary component may not be the sole cause or solution. Luteolin is a naturally occurring compound with potential benefits for migraine management due to its anti-inflammatory and antioxidant properties. Although it may assist certain individuals in effectively managing migraine. This study will provide the pharmacokinetic and toxicity profile of luteolin that may assist researchers while developing any type of formulation and additionally, docking analysis will give information regarding the binding affinity of the CGRP proteins.

2. Materials and methods

2.1. Pharmacokinetic assessment

Luteolin's standard chemical structure information was obtained from the PubChem server. Pharmacokinetic studies were conducted using both pkCSM and Swiss ADME online tool.[45] The ADMET profile (Absorption, Distribution, Metabolism, Excretion, and Toxicity) was retrieved from the host computer, utilizing canonical smiles. Both pkCSM and Swiss ADME offer insights into the drug's pharmacokinetics (PK), pharmacodynamics (PD), and toxicological (Toxicity) characteristics. The web-based application pkCSM was utilized to explore the pharmacokinetic properties of drugs. Luteolin underwent an analysis of its physicochemical properties using the pkCSM (Pires et al., 2015; Dulsat et al., 2023). Based on their binding affinities and pharmacokinetic characteristics, the most favourably docked compounds were pinpointed, highlighting their potential as viable therapeutic candidates. Nevertheless, when implementing effective strategies in drug design, development, and discovery endeavours, it is imperative to encompass a range of crucial pharmacokinetic aspects or ADMET properties, encompassing absorption, distribution, metabolism, excretion, and toxicity. To delve into the physicochemical properties and ADMET attributes of the substance under investigation, a thorough investigation was conducted using web-based tools. Luteolin underwent an examination based on Lipinski's rule of five, which identifies five fundamental physicochemical characteristics known to significantly influence a molecule's efficacy, safety, or metabolic behavior. In the process, violations of LogP were detected across all three Consensus LogPo/w models that were assessed, specifically, WLOGP, XLOGP3, and LOGP. The logarithm of the partition coefficient between n-octanol and water was determined to gauge lipophilicity values ranging from -0.7 to $+5.0$, utilizing atomistic and topological implementations of Moriguchi's topological approach, including XLOGP3, WLOGP, and MLOGP.[46,47]

2.2. Toxicological modelling and simulation

To avert potential complications that may arise during the withdrawal of a drug, including the risk of organ system failure or harm, it is imperative to conduct toxicological assessments. The OSIRIS Property Explorer online tool[48] was employed in conjunction with PubChem

structures to assess the toxicity levels of Luteolin. These substances were ranked using a color-coded scale to determine their potential for causing cancer, inducing mutations, provoking irritation, affecting reproductive function, and serving as a potential pharmaceutical agent with high-risk substances represented in red, moderate risk in yellow, and low-risk compounds in green. The assessment involved the computation of an overall drug score, a drug-likeness score, and the Topological Polar Surface Area (TPSA) based on specific toxicity criteria.[49,50]

2.3. Molecular property analysis

The Luteolin were subject to in silico testing via Molinspiration on-line tool[51] to assess drug similarity and forecast bioactivity. The likelihood of the molecule under consideration exhibiting activity increases with a higher score. A compound with a bioactivity score exceeding 0.00 is deemed to possess noteworthy biological properties. In the bioactivity range, scoring between −0.50 and 0.00 indicates a high level of activity, whereas values falling below −0.50 are indicative of inactivity.[48,52]

2.4. Molecular docking analysis

The data source for our docking analysis primarily consists of the molecular structures of both the ligands and the target proteins involved. These structures were obtained from publicly available databases such as the Protein Data Bank such as 6ZHO, 6PFO, 7P0I, 6PGQ, 6E3Y, 6NIY, and 7KNU (accessible at http://www.rcsb.org). These protein structures were chosen based on their relevance to the biological pathways or mechanisms associated with the condition under investigation, in this case, migraine therapy. The extracted PDB structures underwent refinement through the removal of inhibitor ligands and any other associated chains. Furthermore, the ligands used in the docking analysis were sourced from a variety of databases, including but not limited to the ZINC database, PubChem, and ChEMBL. These databases provide a vast collection of small molecules with diverse chemical structures, allowing us to explore a wide range of potential ligands for their binding affinity to the target proteins. The proteins utilized in this investigation were engineered with assistance from AutoDockTools v1.5.6.[53] Subsequently, a secondary refinement round was conducted on the modified proteins to stabilize the ionized and tautomeric states of the amino acid residues. This process entailed the removal of water molecules and the addition of hydrogen atoms. To maintain accessibility for docking research, a PDB file was generated to house the updated protein structures. The optimization of these proteins was carried out with the assistance of the Molegro molecular viewer. Alterations to bond orders were implemented when removing water and covalently attached ligands. After assigning charge and protonation states, energy minimization was performed using a molecular mechanics force field. The AutoDock software was employed to calculate Gasteiger charges and assess ligand rotatable bonds, thus generating multiple conformers for the docking process. Receptor grids were constructed based on receptor-specific points, and grid boxes were established using the axes of these receptor grids as the coordinate system. The grid parameters XYZ dimensions kept as default to 40 as well as spacing 0.375A°, and attributes noted as X −19.409600; Y 74.650750; Z 33.849550. Various maps were created using Autogrid 4. All molecular docking simulations were executed utilizing the Lamarckian genetic algorithm. The parameters used for the Lamarckian genetic algorithm were carefully chosen to optimize the efficiency and accuracy of the docking process. The mutation rate determines the probability of a mutation occurring during the genetic algorithm optimization process. In our analysis, we set the mutation rate to 0.02. The crossover rate determines the probability of crossover events, where genetic information is exchanged between parent chromosomes, during the genetic algorithm optimization process. In our analysis, we set the crossover rate to 0.8. The population size refers to the number of individuals (chromosomes) in each generation of the genetic algorithm. A larger population size allows for a more thorough exploration of the conformational space but may require more computational resources. In our analysis, we set the population size to 150. The docking process was configured with the following parameters: 50 iterations, 150 subjects, 2.5 million evaluations, and 27,000 generations. Biovia Discovery Studio 2021 facilitated the importation of docking snapshots, and the resulting docked structures were exported in pdbqt format. Subsequently, Biovia Discovery Studio 2021[54] was employed for the visualization of docking results, revealing the presence of hydrogen and hydrophobic contacts at the inhibitor binding sites of the docked target proteins.[55,56]

2.5. Assessment of luteolin targets associated

The search for "Migraine" in DisGeNet (https://www.disgenet.org/) yielded lists of genes, which were then combined and duplicates were removed. Subsequently, the data was standardized using the UniProt database to identify relevant targets for migraine treatment. To pinpoint the targets relevant to herb-based migraine therapy, a Venn diagram was created using data from Bioinformatics and Evolutionary Genomics (https://bioinformatics.psb.ugent.be/webtools/Venn/). This allowed us to identify the common targets associated with Luteolin in migraine treatment.

2.6. Biomolecule-Target-Pathway network building (B-T-P)

To ascertain the suitability of Luteolin for treating migraines, we inputted its active constituents, potential targets, and the leading 20 pathways into the Cytoscapev_3.9.1 software.[57] Within this software, we computed topological parameters for the network, such as degree, betweenness, and proximity. Utilizing the Network Analyzer tool, we then identified the key target and the most influential active biomolecules within Luteolin for migraine treatment.

3. Results

3.1. Pharmacokinetic evaluation of luteolin

The pharmacokinetic data of luteolin reveals key properties relevant to pharmacology, toxicology, and drug development. With low water solubility (−3.094), it may not easily dissolve in water and has limited permeability (0.096) through intestinal cell layers. However, it shows good intestinal absorption (81.13), suggesting effective absorption in the gastrointestinal tract. It has low skin permeability (−2.735) and is a substrate for P-glycoprotein. The volume of distribution (Vdss) is 1.153, indicating its distribution in the body, with 0.168 fractions unbound in the bloodstream affecting distribution and activity. Luteolin exhibits limited blood-brain barrier permeability (−0.907) and CNS permeability (−2.251), indicating poor penetration into the brain. It is not a substrate for CYP2D6 and CYP3A4 but inhibits CYP1A2 and CYP2C9 is listed in Table 1. These properties affect its metabolism and potential drug interactions. Luteolin's total clearance value (0.495) reflects its elimination rate, and it is not a substrate for renal organic cation transporter 2 (OCT2).

3.2. Toxicological profile of luteolin

Compound analysis indicates moderate lipophilicity (cLogP = 1.99), potentially impacting bioavailability and distribution. Low solubility (−2.56) suggests limited water solubility, affecting practical use. Moderate polarity (TPSA = 107.22 Å^2) is mentioned in Table 2 suggesting suitability for drug development (value = 1.9). With 6 hydrogen bond acceptors and 4 donors, the compound exhibits potential for intermolecular interactions. Limited flexibility is indicated by 1 rotatable bond. A drug score of 0.88 suggests potential as a drug candidate. The predicted human maximum tolerated dose is 0.499; rat oral acute toxicity (LD50) is 2.455, and chronic toxicity (LOAEL) is 2.409. Toxicity values

to T. Pyriformis (0.326) and minnows (3.169) indicate potential aquatic toxicity. Not an inhibitor of hERG I or hERG II, relevant for cardiac safety. "No toxicity" for mutagenic, tumorigenic, irritant, and reproductive effects, though comprehensive toxicological assessments are warranted for safety evaluation.

3.3. Exploration of Luteolin's impact on KEGG pathways in managing migraine

We conducted searches in DisGeNet 512 gene databases to identify targets related to migraines. Upon comparing these migraine-related targets to the 104 targets associated with Luteolin, we identified 17 common targets represented in Fig. 1. Subsequently, we performed protein-protein interaction analysis on these shared targets. This analysis focused on assessing enrichment in terms of Gene Ontology (GO) and KEGG pathways. The results of our investigation included a comprehensive total of 161 GO enrichment findings, encompassing cellular component analysis (01), biological process analysis (150), and molecular function analysis (10) illustrated in Fig. 2. We extracted the results related to the top ten GO characteristics and stored and visualized these findings using a bioinformatics platform. In terms of KEGG analysis, we identified a total of 22 pathways. The majority of these KEGG pathways encompass processes such as Endocrine resistance, Estrogen signaling pathway, TNF signaling pathway, Prolactin signaling pathway, Proteoglycans in cancer, IL-17 signaling pathway, Prostate cancer, Serotonergic synapse, Relaxin signaling pathway, and Dopaminergic synapse.

3.4. Enrichment analysis of biomolecule target pathway network

We created a network diagram, known as the Biomolecule-Target-Pathway (B-T-P) diagram, to illustrate in Fig. 3 the interactions between biomolecules, targets, and pathways involved in the treatment of migraines by Luteolin. This diagram was constructed using CytoScape 3.9.1. To identify the components and primary target actions within this network, we analyzed its topological properties using integrated tools. The network comprises 601 nodes connected by 616 edges, as depicted. It's worth noting that the Luteolin exhibits substantial potential for the treatment of migraines. Among the topological parameters, the top 5 targets identified were PTGS2, AKT1, ESR1, MMP2, and MMP9 of degree scores 12, 12, 10, 09, and 08. These targets hold significant promise as candidates for Luteolin in the context of migraine treatment. The top 10 shortest path targeted proteins of migraine and Luteolin were found to be AKR1A1, MAOA, MMP2, ABCB1, BACE1, APP, MMP9, ACHE, ESR2, and CYP19A1. The neuroinflammatory response during a

migraine attack could be regulated by targeting specific proteins like MMP3, MMP9, and PTGS2. Additionally, other proteins outlined in Table 3 might serve as novel targets for migraine treatment. Conducting focused research on each of these proteins is essential for advancing migraine therapies. Concentrating on neuroinflammatory markers like MMP3, MMP9, and PTGS2 may be linked to migraine attacks is illustrated in Fig. 4 (see Table 3).

3.5. Docking assessment of luteolin targeting different CGRP proteins

To determine whether Luteolin might directly bind to the chosen genes, a ligand binding simulator was run before the experimental study. CGRP protein and Luteolin showed the highest binding energy in PDB: $6PFO = -7.63$ kcal/mol formed a hydrogen bond with amino acid residues THR127, THR75, TYR71; and other subsequent docking score mentioned as follows $7KNU = -6.52$ kcal/mol formed a hydrogen bond with amino acid residues ILE106, TRP56; $6PGQ = -5.65$ kcal/mol formed a hydrogen bond with amino acid residues SER129, LYS110; $6NIY = -5.42$ kcal/mol formed a hydrogen bond with amino acid residues ASN233, HIS156, GLN383; $6E3Y = -5.18$ kcal/mol formed a hydrogen bond with amino acid residues THR131, ASP90. Since molecular docking persists as a computer simulation approach used for determining the shape of a receptor ligand complex, the hypothesis desires to be tested empirically in future studies, but the data presented here suggests that Luteolin might have a significant ability to directly connect with CGRP. Each target ligand generated at least one hydrogen bond with active chemical residues, providing confidence in the accuracy and precision of the prediction made by the investigation. In most selected targets, a considerably increased number of amino acid residues engaged in hydrogen bonding and van der Waals interactions was related to shorter hydrogen bond lengths (less than 3.0).

4. Discussion

Neuroinflammation has been increasingly recognized as a key player in the pathophysiology of migraine headaches, although the exact mechanisms are still not fully understood. Migraine is a complex neurological disorder characterized by recurrent episodes of moderate to severe headaches, often accompanied by sensory disturbances, nausea, and other symptoms.[58] The identified targets (PTGS2, AKT1, ESR1, MMP2, and MMP9) play significant roles in MAPK and NFkB pathways. PTGS2, also known as COX-2, is involved in inflammatory processes and is a target for Non-steroidal anti-inflammatory drugs (NSAIDs), commonly used in migraine management. AKT1 is implicated in modulating inflammatory responses. In migraine, neuroinflammation is considered a contributing factor, and AKT1 may influence the release of inflammatory mediators within the central nervous system. AKT1 has been associated with the regulation of vascular tone. As changes in blood vessel diameter are characteristic of migraines, AKT1 may have a role in modulating the vascular aspects of migraine attacks. Estrogen has been recognized as a key factor in migraine, and its fluctuations, especially during the menstrual cycle, can trigger or exacerbate migraines in susceptible individuals, often affecting women more than men. ESR1, being a receptor for estrogen, is involved in mediating the effects of estrogen in various tissues, including those relevant to migraine pathophysiology. Targeting ESR1 may offer potential avenues for hormonal modulation in the management of migraines, especially in cases where hormonal fluctuations contribute to the occurrence of headaches. MMP2 and MMP9 are involved in matrix remodelling, suggesting a potential role in migraine-associated neurovascular changes. Elevated levels of MMP2 and MMP9 have been reported in conditions involving neuroinflammation. In migraine, neuroinflammatory processes are thought to contribute to the initiation and propagation of attacks. MMP2 and MMP9, have been linked to the mechanisms underlying CSD.[59] Understanding the significance of these targets provides insights into potential therapeutic strategies for migraine treatment. In our interpretation,
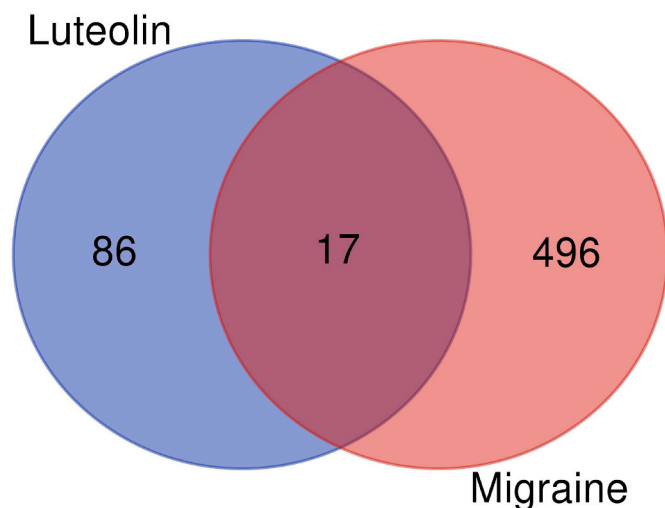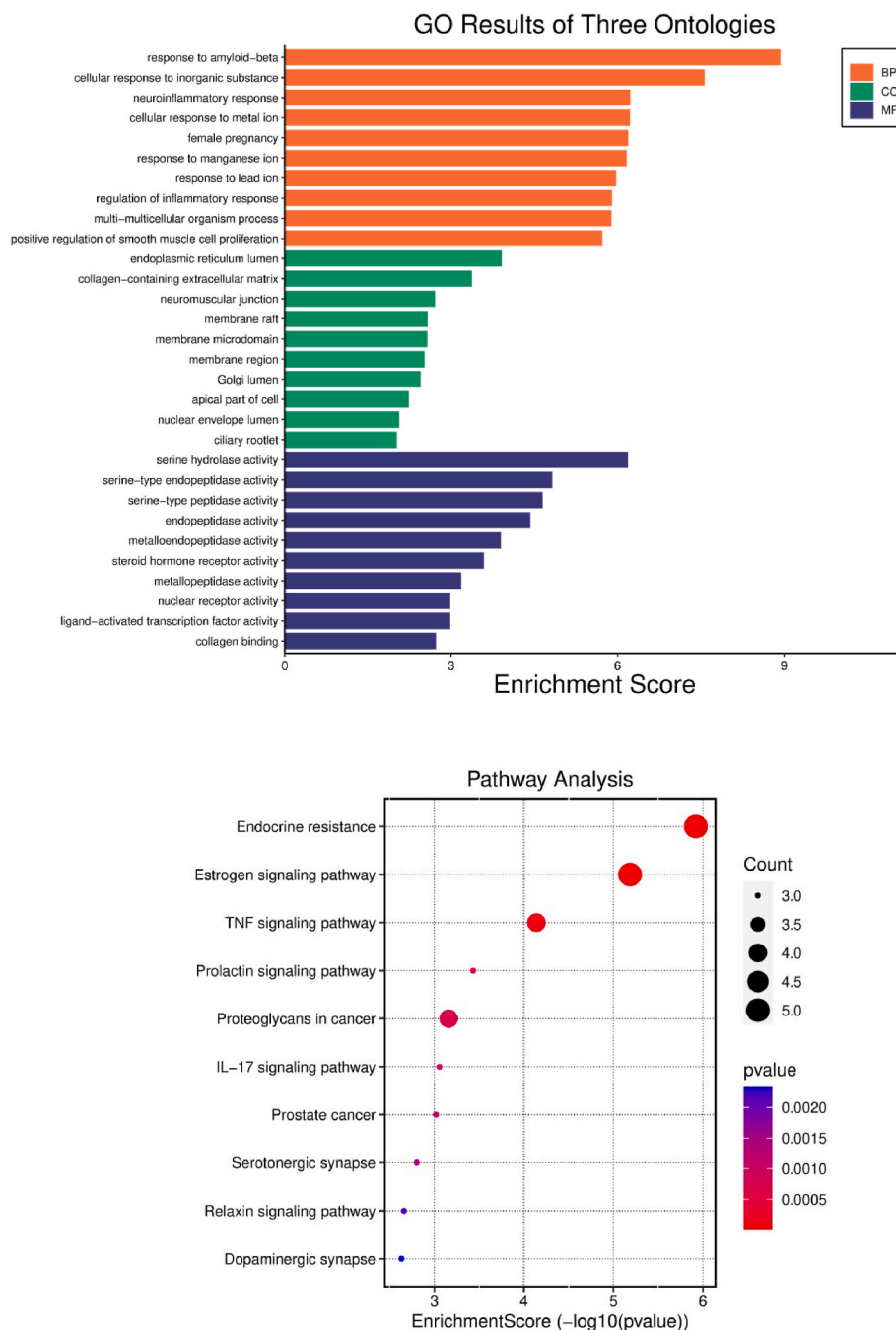

Fig. 1. Common Targets of Luteolin and migraine.

Fig. 2. Gene otology and KEGG enrichment analysis.

several specific aspects of luteolin contribute to its promise for migraine management. Firstly, luteolin possesses anti-inflammatory properties, which can help alleviate neuroinflammation, a key component in migraine pathophysiology. Secondly, luteolin acts as an antioxidant, potentially reducing oxidative stress, which has been implicated in migraine attacks. Additionally, luteolin has been shown to modulate various signaling pathways involved in pain perception and neuronal excitability, offering a multifaceted approach to migraine relief. Furthermore, luteolin's ability to inhibit pro-inflammatory cytokines and block pain receptors may provide further therapeutic benefits in migraine management. Overall, these properties underscore luteolin's potential as a natural remedy for mitigating migraine symptoms. Neuroinflammatory mediators, including cytokines such as IL-1β, IL-17, CGRP, and TNFα can sensitize and activate trigeminal nociceptive

pathways, which are involved in transmitting pain signals from the head and face to the brain.[60] This activation contributes to the generation and propagation of migraine pain. During a migraine attack, there is evidence of increased levels of pro-inflammatory mediators such as cytokines, chemokines, and prostaglandins in the trigeminal system and meninges. These molecules can sensitize nociceptive neurons and promote neurogenic inflammation, leading to pain and other migraine symptoms. Neuroinflammation can lead to dysfunction of the blood-brain barrier, allowing peripheral inflammatory mediators to penetrate the brain parenchyma. This can trigger a cascade of inflammatory responses within the brain, contributing to migraine pathophysiology.[61] Glial cells, particularly microglia and astrocytes, play a crucial role in neuroinflammatory processes. Activation of glial cells in response to various stimuli, such as stress or inflammation, can release
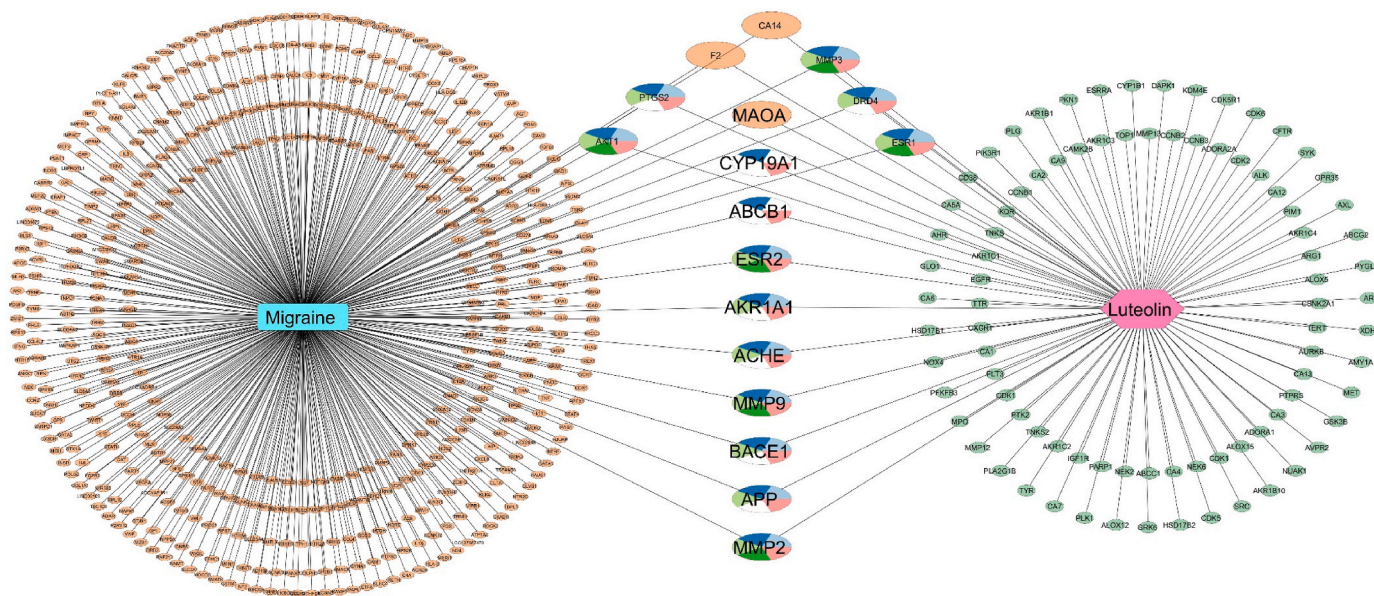
Fig. 3. Common target proteins of Luteolin and Migraine.

Table 1
Pharmacokinetic profile of Luteolin.

Property	Model Name	Predicted Value
Absorption	Water solubility	−3.094 log mol/L
Absorption	Caco2 permeability	0.096 log Papp in 10^{-6} cm/s
Absorption	Intestinal absorption (human)	81.13 %
Absorption	Skin Permeability	−2.735 log Kp
Absorption	P-glycoprotein substrate	Yes
Absorption	P-glycoprotein I inhibitor	No
Absorption	P-glycoprotein II inhibitor	No
Distribution	VDss (human)	1.153 log L/kg
Distribution	Fraction unbound (human)	0.168 Fu
Distribution	BBB permeability	−0.907 log BB
Distribution	CNS permeability	−2.251 log PS
Metabolism	CYP2D6 substrate	No
Metabolism	CYP3A4 substrate	No
Metabolism	CYP1A2 inhibitior	Yes
Metabolism	CYP2C19 inhibitior	No
Metabolism	CYP2C9 inhibitior	Yes
Metabolism	CYP2D6 inhibitor	No
Metabolism	CYP3A4 inhibitor	No
Excretion	Total Clearance	0.495 log ml/min/kg
Excretion	Renal OCT2 substrate	No
Toxicity	AMES toxicity	No
Toxicity	Max. tolerated dose (human)	0.499 log mg/kg/day
Toxicity	hERG I inhibitor	No
Toxicity	hERG II inhibitor	No
Toxicity	Oral Rat Acute Toxicity (LD50)	2.455 mol/kg
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	2.409 log mg/kg_bw/day
Toxicity	Hepatotoxicity	No
Toxicity	Skin Sensitization	No
Toxicity	T.Pyriformis toxicity	0.326 log ug/L
Toxicity	Minnow toxicity	3.169 log mM

Table 2
Physiochemical parameters, lipophilicity and water solubility of Luteolin.

Molecule	Luteolin
Canonical SMILES	Oc1cc(O)c2c(c1)oc(cc2 = O)c1ccc(c(c1)O)O
Formula	C15H10O6
MW	286.24 g
Heavy atoms	21
Aromatic heavy atoms	16
Fraction Csp3	0
Rotatable bonds	1
H-bond acceptors	6
H-bond donors	4
MR	76.01
TPSA	107.22
cLOGP	1.99
XLOGP3	2.53
WLOGP	2.28
MLOGP	−0.03
Silicos-IT Log P	2.03
Consensus Log P	1.73
ESOL Log S	−3.71
ESOL Solubility	5.63E-02 mg/ml
ESOL Solubility	1.97E-04 mol/l
ESOL Class	Soluble
Ali Log S	−4.51
Ali Solubility	8.84E-03 mg/ml
Ali Solubility	3.09E-05 mol/l
Ali Class	Moderately soluble
Silicos-IT LogSw	−3.82
Silicos-IT Solubility	4.29E-02 mg/ml
Silicos-IT Solubility	1.50E-04 mol/l
Silicos-IT class	Soluble

pro-inflammatory cytokines and neurotransmitters, amplifying pain signaling pathways and contributing to migraine attacks. Neuro-inflammation can lead to central sensitization, a process characterized by increased excitability of neurons in the central nervous system.[62] Central sensitization is believed to underlie the development of chronic pain conditions, including migraine. Persistent activation of nociceptive pathways and alterations in synaptic transmission contribute to the amplification and prolongation of migraine pain. There is evidence to suggest that genetic factors predispose individuals to neuro-inflammatory responses and migraine susceptibility. Environmental factors such as stress, sleep disturbances, dietary triggers, and hormonal

fluctuations can also modulate neuroinflammatory pathways and trigger migraine attacks in susceptible individuals.

Tumor necrosis factor-alpha (TNF-α) is a cytokine that plays a significant role in neuroinflammation, which is the inflammation of the nervous tissue. The TNF-α signaling pathway is one of the key pathways involved in regulating the inflammatory response in the central nervous system (CNS). TNF-α exerts its effects by binding to its receptors, TNFR1 and TNFR2. TNFR1 is expressed ubiquitously and is involved in mediating most of the pro-inflammatory effects of TNF-α, including apoptosis and inflammation. TNFR2 is expressed predominantly in immune cells and is implicated in tissue regeneration and repair. The binding of TNF-α

Table 3
Enrichment analysis of biomolecule target pathway.

Regulation of Neuroinflammatory response	Regulation of inflammatory response	Nitric oxide synthase regulatory activity	TNF signaling Pathway	Prolactin signaling Pathway
MMP2	MMP2	AKT1	APP	ESR1
	MMP9			
MMP9	PTGS2		MMP2	
	APP	ESR1		ESR2
PTGS2	CYP19A1		MMP9	
	ESR1		PTGS2	

to its receptors leads to the activation of downstream signaling cascades. TNFR1 activation can lead to the recruitment of adaptor proteins such as TNF receptor-associated death domain (TRADD), which initiates the formation of a complex called the TNF receptor complex I.[63] One of the major pathways activated downstream of TNFR is the nuclear factor-kappa B (NFkB) pathway. In the canonical NFkB pathway, activation of TNFR leads to the activation of IkB kinase (IKK), which phosphorylates inhibitory proteins of NFkB (IkBs), leading to their degradation. This allows NFkB to translocate into the nucleus and initiate the transcription of pro-inflammatory genes, including cytokines, chemokines, and adhesion molecules. TNFα also activates MAPK pathways, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK.[64] These pathways are involved in the regulation of various cellular processes such as cell proliferation, differentiation, and apoptosis. Activation of MAPKs by TNF-α contributes to the induction of pro-inflammatory gene expression. Production of Activation of NFkB and MAPK pathways leads to the production and release of various inflammatory mediators such as interleukins (IL-1, IL-6), chemokines (IL-8, MCP-1), and prostaglandins. These mediators further amplify the inflammatory response and recruit immune cells to the site of inflammation in the CNS. Interleukin-17 (IL-17) is a cytokine that plays a significant role in inflammation and immune responses. It is produced by various cells, including T-helper 17 (Th17) cells, γδ T cells, natural killer T cells, and some innate immune cells like neutrophils and mast cells. IL-17 binds to its receptor complex, composed of IL-17RA and IL-17RC, which are expressed on various cells within the CNS, including astrocytes, microglia, and endothelial cells is illustrated in Fig. 5. Upon binding of IL-17 to its receptor, several intracellular signaling pathways are activated, including the NFkB and MAPK pathways.[65] Activation of these pathways leads to the upregulation of proinflammatory cytokines, chemokines, and other inflammatory mediators. These include IL-6, IL-1β, TNF-α, CXCL1, CXCL2, and CXCL8, among others. Simultaneously, IL-1β is initially synthesized as an inactive precursor protein (pro-IL-1β). Upon activation of microglia, pro-IL-1β is cleaved by an enzyme called caspase-1 into its active form, IL-1β. Binding to IL-1 receptors: Once released, IL-1β binds to its receptors, IL-1 receptor type 1 (IL-1R1) and IL-1 receptor accessory protein (IL-1RAcP), which are expressed on various cell types including neurons, astrocytes, and endothelial cells within the CNS. The binding of IL-1β to its receptors initiates intracellular signaling cascades, primarily through the activation of the NFkB and the MAPK pathways.[66] These pathways lead to the activation of transcription factors and induction of gene expression of various pro-inflammatory molecules, including additional cytokines, chemokines, and adhesion molecules. Calcitonin gene-related peptide (CGRP) is a neuropeptide that plays a significant role in migraine headache pathophysiology. During a migraine attack, trigeminal sensory nerves release CGRP in response to various triggers such as stress, certain foods, hormonal changes, or environmental factors. CGRP causes vasodilation of blood vessels in the brain, leading to an increase in blood flow. This vasodilation is believed to contribute to the throbbing pain experienced during migraine headaches. CGRP also sensitizes trigeminal neurons, increasing their responsiveness to other pain-inducing stimuli. This sensitization can lower the threshold for pain perception, making

individuals more susceptible to experiencing migraine attacks. CGRP has pro-inflammatory effects, promoting the release of other inflammatory mediators and contributing to the neurogenic inflammation seen in migraine headaches.[67] CGRP acts as a neurotransmitter or neuromodulator in the pain pathway, transmitting pain signals from the trigeminal nerves to higher brain centers involved in pain processing. CGRP may also play a role in migraine aura, the sensory disturbances that some individuals experience before or during a migraine attack. CGRP release may contribute to the changes in cortical excitability and neuronal function associated with aura symptoms.[7] The interlinking mechanisms involving CGRP, IL-1β, TNF-α, NFkB, IL-17, and MAPK pathways contribute to the neuroinflammatory processes underlying migraine headache. Targeting these pathways with specific inhibitors or monoclonal antibodies has emerged as a promising therapeutic approach for migraine prevention and treatment. Herbal remedies may offer potential benefits for migraine management.

Herbal therapy shows promise for managing migraine headaches, with certain remedies known for their anti-inflammatory, analgesic, and neuroprotective properties. Luteolin, identified through compound screening, emerges as a potential candidate for migraine therapy due to its anti-inflammatory and neuroprotective effects. This flavonoid, found in fruits, vegetables, and medicinal herbs, holds the potential for alleviating migraine symptoms. Luteolin-rich foods, such as celery and parsley, can be integrated into a migraine-friendly diet as they contain potential anti-inflammatory properties and may help mitigate the risk of triggering attacks. However, it's important to note that individual responses to dietary changes can vary, and a comprehensive approach considering various triggers is recommended for effective migraine. The mutagenicity, tumorigenicity, irritability, and reproductive effects indicate that the compound exhibits a significant toxicity profile for the luteolin. Luteolin does not function as an inhibitor for hERG I or hERG II. Our research delves into the pharmacokinetics of luteolin, focusing on its absorption, distribution, metabolism, and excretion in the body. Understanding these processes is crucial for developing effective formulations and optimizing dosage regimens for migraine prevention. While luteolin is generally safe in dietary amounts, its toxicity profile needs evaluation for concentrated forms or supplements. Properties like its cLogP value (1.99), solubility, and TPSA (107.22) offer insights into its bioavailability and distribution. These factors influence absorption, circulation, and interaction with biological components, essential for their therapeutic efficacy. Topological analysis identified PTGS2, AKT1, ESR1, MMP2, and MMP9 as promising targets. The findings indicate significant interactions between luteolin and calcitonin gene-related peptide (CGRP) proteins shown a binding affinity of −7.63 kcal/mol. The robust absorption suggests that luteolin could be efficiently delivered through oral routes, facilitating convenient and effective incorporation into therapeutic strategies for managing migraines. It has been studied for its potential anti-inflammatory properties and its ability to modulate signaling pathways involved in inflammation, such as the MAPK and NFkB pathways.[68] These pathways play key roles in the regulation of inflammatory responses, including those associated with migraine headaches. NFkB is a transcription factor that regulates the expression of many pro-inflammatory genes. Luteolin has been shown to inhibit the activation of NFkB by blocking the phosphorylation and degradation of its inhibitory protein, IkBα, thus preventing its translocation into the nucleus and subsequent induction of pro-inflammatory gene expression. MAPKs are a family of protein kinases involved in the regulation of cellular responses to various stimuli, including inflammation. Luteolin has been reported to inhibit the phosphorylation and activation of MAPKs, such as p38, ERK, and JNK thereby attenuating downstream inflammatory signaling cascades. By downregulating the activity of these pathways, luteolin can reduce the production of pro-inflammatory molecules, including cytokines, chemokines, and adhesion molecules, which contribute to neuroinflammation associated with migraine headaches. Regarding migraine therapy, targeting neuroinflammation has emerged as a promising approach for the treatment
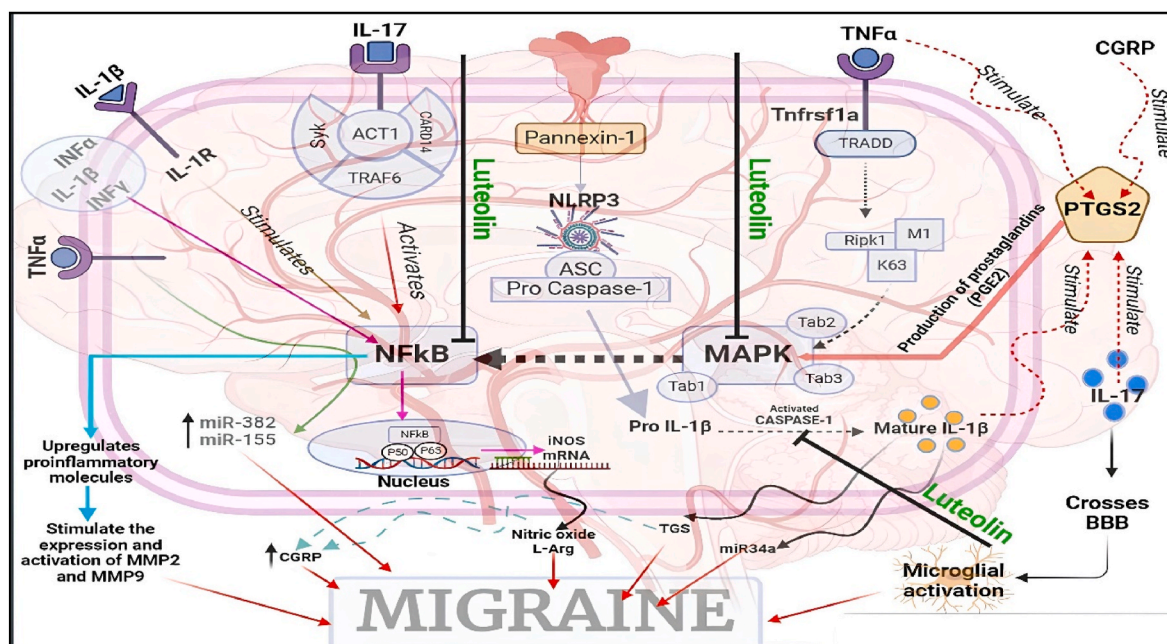
Fig. 4. The docking interactions between Luteolin and various CGRP proteins.

and prevention of migraine attacks. Since neuroinflammation plays a significant role in the pathophysiology of migraines, substances like luteolin which possess anti-inflammatory properties and can modulate key inflammatory pathways may have potential therapeutic benefits. However, it's essential to note that while preclinical studies have shown promising results regarding luteolin's anti-inflammatory effects, further research is needed to validate its efficacy and safety for migraine therapy in clinical settings. Additionally, the bioavailability and pharmacokinetics of luteolin need to be considered to ensure its effectiveness in vivo. Nevertheless, luteolin and other natural compounds with similar

properties represent a promising area for future drug development in the treatment of migraine and other inflammatory conditions.

5. Future perspective

Luteolin shows promise for migraine treatment due to its neuroprotective properties. Further research on its mechanisms, dosage, and interactions with existing treatments is needed. Long-term clinical trials in diverse patient groups would provide valuable insights for migraine management and other neurological disorders Further research and

Fig. 5. Interplay of NFkB and MAPK signaling pathways in migraine pathophysiology.

clinical trials are warranted to validate its efficacy, safety, and optimal dosage for migraine treatment. However, further research is needed to better understand the efficacy, safety, and mechanisms of action of herbal treatments for migraine, as well as their potential interactions with conventional medications. We plan to conduct in-depth toxicological studies that extend beyond the initial assessments. Additionally, we recognize the importance of investigating specific populations, such as different age groups and individuals with pre-existing conditions, in our safety studies to ensure a more nuanced understanding of luteolin's safety profile across diverse demographics. This comprehensive approach will enhance the reliability and applicability of our findings. Developing nanoformulations for luteolin can enhance its solubility and permeability, particularly across the blood-brain barrier, promising improved bioavailability and efficacy for migraine and other neurological conditions. Further research is needed to elucidate the molecular mechanisms of luteolin's interaction with CGRP and its role in modulating neuroinflammation, aiding in the development of targeted therapies. Given the complexity of migraine, personalized medicine approaches should be explored to tailor treatments based on individual patient factors. Investigating luteolin in combination with existing migraine medications or therapies may yield synergistic effects, improving treatment outcomes while minimizing side effects. Additionally, considering luteolin's broader public health impact, including its potential to alleviate the economic and societal burden of migraines, is essential.

6. Conclusion

In conclusion, neuroinflammation plays a significant role in the pathophysiology of migraine headaches, with various molecular targets implicated in the MAPK and NF-κB pathways. These targets include PTGS2, AKT1, ESR1, MMP2, and MMP9, each contributing to the inflammatory processes underlying migraine attacks. Herbal remedies, such as luteolin, have shown promise in managing migraines due to their anti-inflammatory and neuroprotective properties. Luteolin's ability to modulate signaling pathways involved in pain perception and neuronal excitability offers a multifaceted approach to migraine relief. Moreover, luteolin's potential to inhibit pro-inflammatory cytokines and block pain receptors further supports its therapeutic benefits in migraine management. Understanding the interplay between neuroinflammatory

mediators like CGRP, IL-1β, TNF-α, and the pathways they activate provides insights into potential therapeutic strategies for migraine treatment. Although luteolin demonstrates promising anti-inflammatory effects in preclinical studies, further research is warranted to validate its efficacy and safety for migraine therapy in clinical settings. Nevertheless, luteolin and similar natural compounds represent a promising avenue for future drug development in the treatment of migraine and other inflammatory conditions.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Rapuru Rushendran; Interpreted, data analysis, supervised, validation, and data compilation by Vellapandian Chitra. The first draft of the manuscript was written by Rapuru Rushendran and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

There are no conflicts of interest between the writers and the manuscript's content, and the authors have no relevant relationships with any organizations related to the topic or materials covered. This covers any and all salaries, bonuses, commissions, honoraria, stock options, expert witness fees, grants, patents, and royalties.

Funding

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:
Chitra Vellapandian reports were provided by SRM Institute of Science and Technology (Deemed to be University). If there are other authors, they declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

R RUSHENDRAN reports financial support was provided by India Ministry of Tribal Affairs. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

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