Evaluation of Membrane Stabilizing Activity and Protein Denaturation Activity of Hesperetin

[1]Sapana A. Kuppekar, [2]Suraj H. Kolekar, [2]Akanksha A. Gadekar and [1]Atul R. Chopade,
[1] *Department of Pharmacology, KE's Rajarambapu College of Pharmacy, Kasegaon, Taluka- Walawa, District- Sangli 415404. Maharashtra, India.*
[2]*Department of Pharmaceutical Quality Assurance, KE's Rajarambapu College of Pharmacy, Kasegaon, Taluka- Walwa, District- Sangli 415404. Maharashtra, India.*

Abstract: **This study investigated the membrane stabilizing activity and protein denaturation inhibition properties of hesperetin, a flavanone predominantly found in citrus fruits. Using established in vitro models, we evaluated hesperetin's ability to prevent hypotonic solution-induced and heat-induced erythrocyte hemolysis, as well as heat-induced protein denaturation. Hesperetin demonstrated significant concentration-dependent membrane stabilization, with maximum inhibition of 73.6% at 200 µg/mL in the hypotonic solution model and 70.8% at 200 µg/mL in the heat-induced model, comparable to diclofenac sodium (78.4% and 75.2%, respectively). In protein denaturation assays, hesperetin exhibited notable inhibitory activity with an IC50 value of 57.4 µg/mL, while diclofenac sodium showed an IC50 of 42.6 µg/mL. Molecular docking studies revealed favorable interactions between hesperetin and membrane phospholipids (binding energy: -7.3 kcal/mol), as well as specific binding to bovine serum albumin (binding energy: -8.1 kcal/mol). The hydroxyl groups at positions 5, 7, and 3' of hesperetin formed multiple hydrogen bonds with target molecules, contributing to its stabilizing effects. These findings provide evidence for hesperetin's significant membrane protective and protein stabilizing properties, supporting its potential as a therapeutic agent for inflammatory conditions. Further in vivo studies are warranted to validate these effects and explore clinical applications.**

Keywords: **Bovine serum albumin, Diclofenac sodium, Hesperetin, Membrane stabilizing activity, Molecular docking, Protein denaturation.**

INTRODUCTION

Inflammation represents a complex biological response to harmful stimuli and is implicated in the pathogenesis of numerous disorders including rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, and various neurodegenerative conditions.[1] While acute inflammation is a protective response essential for tissue repair, chronic inflammation can lead to progressive tissue damage and dysfunction.[2]

Current pharmacological approaches to manage inflammatory conditions predominantly rely on non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying antirheumatic drugs (DMARDs). However, these therapeutic options are associated with significant adverse effects including gastrointestinal ulceration, cardiovascular complications, and immunosuppression.[3] These limitations have stimulated extensive research into bioactive compounds from natural sources with potential anti-inflammatory properties and improved safety profiles.

Flavonoids constitute a diverse group of polyphenolic compounds widely distributed in fruits, vegetables, and medicinal plants. These phytochemicals have attracted considerable attention due to their broad spectrum of pharmacological activities, including anti-inflammatory, antioxidant, and immunomodulatory effects.[4]

Among flavonoids, hesperetin (3',5,7-trihydroxy-4'-methoxyflavanone) is a prominent flavanone found abundantly in citrus fruits, particularly oranges and lemons.[5]

Previous studies have reported various biological activities of hesperetin, including antioxidant, anti-inflammatory, anti-cancer, cardioprotective, and neuroprotective effects.[6] While its anti-inflammatory activity has been documented, the specific mechanisms underlying this effect remain incompletely understood. Membrane stabilization and inhibition of protein denaturation represent important anti-inflammatory mechanisms by which many therapeutic agents exert their effects.[7]

The erythrocyte membrane is analogous to the lysosomal membrane, and its stabilization can be correlated with the inhibition of release of

inflammatory mediators.[8] During inflammation, lysosomal enzymes are released into the cytosol, resulting in tissue damage, cellular injury, and various inflammatory disorders. Stabilization of the lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents such as proteases and bactericidal enzymes, which cause further tissue inflammation and damage.[9]

Similarly, protein denaturation has been established as a cause of inflammation. Many inflammatory conditions are associated with the production of autoantigens resulting from protein denaturation.[10] Several anti-inflammatory drugs have demonstrated their ability to inhibit thermally-induced protein denaturation, suggesting that prevention of protein denaturation may be one of the mechanisms of their anti-inflammatory action.

Despite growing interest in hesperetin's therapeutic potential, comprehensive evaluations of its membrane stabilizing and protein denaturation inhibition properties remain limited. This study aimed to investigate these specific activities of hesperetin using established in vitro models, compare its efficacy with standard anti-inflammatory agents, and explore potential molecular mechanisms through computational approaches.

Understanding these mechanisms could provide valuable insights into hesperetin's anti-inflammatory potential and support its development as a novel therapeutic agent for inflammatory conditions. Furthermore, this research contributes to the broader understanding of structure-function relationships in flavonoid compounds and their interactions with biological membranes and proteins.

MATERIALS AND METHODS

Chemicals and Reagents

Hesperetin (purity ≥95% by HPLC) was purchased from Yucca Mumbai. Diclofenac sodium was used as a standard anti-inflammatory drug. All other chemicals and reagents used were of analytical grade. Phosphate-buffered saline (PBS, pH 7.4) was freshly prepared before each experiment.

Collection of Blood Samples

Fresh human blood was collected from healthy volunteer Shri Kolekar who is the author of the present article who had not taken any NSAIDs for at least two weeks prior to the experiment. Blood was collected in heparinized tubes and used for membrane stabilization assays within 24 hours of collection.

Preparation of Erythrocyte Suspension

The collected blood was centrifuged at 3000 rpm for 10 minutes. The packed cells were washed three times with isotonic PBS. The volume of packed red blood cells was measured and reconstituted as a 10% v/v suspension with PBS.

Membrane Stabilizing Activity Assay

Hypotonic Solution-Induced Hemolysis

The membrane stabilizing activity was assessed using the method described by Shinde et al. (2019) with minor modifications. The test sample consisted of 1 mL of phosphate buffer (pH 7.4), 2 mL of hypotonic saline (0.36% NaCl), 0.5 mL of erythrocyte suspension, and 0.5 mL of various concentrations of hesperetin (25, 50, 100, 150, and 200 µg/mL in 10% dimethyl sulfoxide). Diclofenac sodium (25, 50, 100, 150, and 200 µg/mL) was used as a standard drug. The control sample contained 0.5 mL of 10% dimethyl sulfoxide instead of the test solution.

The mixtures were incubated at 37°C for 30 minutes and then centrifuged at 3000 rpm for 10 minutes. The hemoglobin content in the supernatant was estimated spectrophotometrically at 560 nm. The percentage inhibition of hemolysis was calculated using the following formula:

% Inhibition of hemolysis = [(OD control - OD test) / OD control] × 100

Where OD control is the optical density of the control sample and OD test is the optical density of the test sample.

Heat-Induced Hemolysis

For heat-induced hemolysis, 5 mL of erythrocyte suspension was mixed with 5 mL of various concentrations of hesperetin or diclofenac sodium (25-200 µg/mL). Control tubes contained 5 mL of erythrocyte suspension and 5 mL of PBS. The mixtures were incubated at 56°C for 30 minutes, followed by cooling under running water. The samples were then centrifuged at 2500 rpm for 5 minutes, and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of hemolysis was calculated as described above.

Protein Denaturation Inhibition Assay

The inhibition of protein denaturation was evaluated according to the method of Kumari et al. (2020) with slight modifications. The reaction mixture (5 mL) consisted of 0.2 mL of bovine serum albumin (1% aqueous solution), 2.8 mL of phosphate buffered saline (PBS, pH 6.4), and 2 mL of various concentrations of hesperetin (12.5, 25, 50, 75, 100, 125, 150, and 200 µg/mL). Diclofenac sodium at the same concentrations was used as a reference standard. The control contained 10% dimethyl sulfoxide instead of the test compound or standard drug.

The samples were incubated at 37°C for 15 minutes followed by heating at 70°C for 5 minutes. After cooling, the turbidity was measured spectrophotometrically at 660 nm. The percentage inhibition of protein denaturation was calculated using the formula:

% Inhibition = [(OD control - OD test) / OD control] × 100

Where OD control is the optical density of the control and OD test is the optical density of the test sample.

Molecular Docking Studies

Molecular docking was performed to investigate potential interactions between hesperetin and membrane phospholipids, as well as bovine serum albumin. The three-dimensional structure of hesperetin was obtained from the PubChem database (CID: 72281) and optimized using Gaussian 09 software with the B3LYP/6-31G basis set. The crystal structure of bovine serum albumin (PDB ID: 4F5S) was retrieved from the Protein Data Bank and prepared using AutoDock Tools 1.5.6 by removing water molecules, adding hydrogen atoms, and computing Gasteiger charges.

A phospholipid bilayer model consisting of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) was constructed using CHARMM-GUI Membrane Builder. Molecular docking simulations were performed using AutoDock Vina with a grid box encompassing the entire protein or the central region of the phospholipid bilayer. The best binding poses were selected based on binding energy values and analyzed using PyMOL and LigPlot+ software.

Statistical Analysis

All experiments were performed in triplicate, and the results are expressed as mean ± standard error of the mean (SEM). Statistical analysis was conducted using GraphPad Prism version 9.0. Differences between groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. P values < 0.05 were considered statistically significant. The IC50 values were calculated using non-linear regression analysis.

RESULTS

Membrane Stabilizing Activity

Hypotonic Solution-Induced Hemolysis

Hesperetin exhibited significant membrane stabilizing activity against hypotonic solution-induced hemolysis in a concentration-dependent manner (Table 1). At the lowest concentration tested (25 µg/mL), hesperetin showed 26.3 ± 1.8% inhibition of hemolysis, which increased progressively with increasing concentrations, reaching a maximum of 73.6 ± 2.9% at 200 µg/mL. The standard drug diclofenac sodium demonstrated slightly higher activity, with inhibition percentages ranging from 31.7 ± 2.1% at 25 µg/mL to 78.4 ± 3.1% at 200 µg/mL.

Table 1. Effect of hesperetin and diclofenac sodium on hypotonic solution-induced hemolysis

Concentration (µg/mL)	% Inhibition of Hemolysis (Mean ± SEM)	
	Hesperetin	Diclofenac sodium
25	26.3 ± 1.8	31.7 ± 2.1
50	38.5 ± 2.2	45.2 ± 2.4
100	56.2 ± 2.5	61.8 ± 2.7
150	67.4 ± 2.7	71.5 ± 2.9
200	73.6 ± 2.9	78.4 ± 3.1
IC50 (µg/mL)	79.3	64.8

The IC50 value for hesperetin was calculated to be 79.3 µg/mL, while diclofenac sodium showed an IC50 of 64.8 µg/mL, indicating that hesperetin possesses comparable membrane stabilizing activity to the standard drug.

Heat-Induced Hemolysis

In the heat-induced hemolysis model, hesperetin also demonstrated significant protection against membrane damage (Table 2). The inhibition of hemolysis ranged from 23.8 ± 1.7% at 25 µg/mL to 70.8 ± 2.8% at 200 µg/mL. Diclofenac sodium showed inhibition percentages from 29.2 ± 2.0% to 75.2 ± 3.0% at the same concentration range.

Table 2. Effect of hesperetin and diclofenac sodium on heat-induced hemolysis

Concentration (µg/mL)	% Inhibition of Hemolysis (Mean ± SEM)	
	Hesperetin	Diclofenac sodium
25	23.8 ± 1.7	29.2 ± 2.0
50	36.4 ± 2.1	42.7 ± 2.3
100	53.7 ± 2.4	58.5 ± 2.6
150	64.9 ± 2.6	68.3 ± 2.8
200	70.8 ± 2.8	75.2 ± 3.0
IC50 (µg/mL)	86.2	72.1

The IC50 value for hesperetin in the heat-induced hemolysis model was 86.2 µg/mL, compared to 72.1 µg/mL for diclofenac sodium. These results indicate that hesperetin effectively protects erythrocyte membranes against both hypotonic and heat-induced damage.

Protein Denaturation Inhibition Activity

Hesperetin demonstrated significant inhibition of heat-induced protein denaturation in a concentration-dependent manner (Table 3). At 12.5 µg/mL, hesperetin showed 18.3 ± 1.5% inhibition of protein denaturation, which increased to 81.7 ± 3.3% at the highest concentration tested (200 µg/mL). The standard drug diclofenac sodium exhibited inhibition percentages ranging from 25.6 ± 1.9% to 86.2 ± 3.5% at the same concentration range.

Table 3. Effect of hesperetin and diclofenac sodium on protein denaturation

Concentration (µg/mL)	% Inhibition of Protein Denaturation (Mean ± SEM)	
	Hesperetin	Diclofenac sodium
12.5	18.3 ± 1.5	25.6 ± 1.9
25	31.6 ± 2.0	38.4 ± 2.2
50	47.2 ± 2.4	53.7 ± 2.5
75	59.8 ± 2.6	65.3 ± 2.7
100	68.5 ± 2.8	73.8 ± 2.9
125	74.2 ± 3.0	78.5 ± 3.1
150	78.4 ± 3.2	82.7 ± 3.3
200	81.7 ± 3.3	86.2 ± 3.5
IC50 (µg/mL)	57.4	42.6

The IC50 value for hesperetin in the protein denaturation inhibition assay was calculated to be 57.4 µg/mL, while diclofenac sodium showed an IC50 of 42.6 µg/mL. These results suggest that hesperetin possesses significant protein stabilizing activity, comparable to the standard anti-inflammatory drug.

Molecular Docking Studies

Molecular docking studies provided insights into the potential interactions between hesperetin and its molecular targets. Docking of hesperetin with the POPC phospholipid bilayer revealed favorable interactions with the polar head groups of phospholipids. Hesperetin exhibited a binding energy of -7.3 kcal/mol and formed hydrogen bonds with the phosphate and choline moieties of POPC molecules, primarily through its hydroxyl groups at positions 5, 7, and 3'. Additionally, hydrophobic interactions were observed between the flavanone backbone of hesperetin and the acyl chains of phospholipids, suggesting potential membrane integration.

Docking studies with bovine serum albumin revealed that hesperetin binds preferentially to subdomain IIA of BSA with a binding energy of -8.1 kcal/mol. The binding site was characterized by a hydrophobic pocket surrounded by polar residues. Hesperetin formed hydrogen bonds with Arg194, Arg198, and His241, while hydrophobic interactions were observed with Leu197, Trp213, and Ala290. The hydroxyl groups at positions 5, 7, and 3' of hesperetin were primarily involved in hydrogen bond formation with protein residues. These interactions may stabilize the protein structure and prevent denaturation under stress conditions.

DISCUSSION

The present study investigated the membrane stabilizing and protein denaturation inhibition properties of hesperetin, providing mechanistic insights into its anti-inflammatory potential. Our findings demonstrate that hesperetin exhibits significant concentration-dependent membrane stabilization against both hypotonic solution-induced and heat-induced hemolysis, as well as notable inhibition of protein denaturation.

Membrane Stabilizing Activity

The erythrocyte membrane is analogous to the lysosomal membrane, and its stabilization can be

correlated with the inhibition of release of inflammatory mediators (Kumar et.al., 2021) When exposed to hypotonic medium or heat stress, the erythrocyte membrane ruptures, releasing hemoglobin. Anti-inflammatory agents can prevent this hemolysis by stabilizing the cell membrane (Chatterjee and Gerlach, 2020).

In our study, hesperetin demonstrated potent membrane stabilizing activity in both hypotonic and heat-induced hemolysis models, with maximum inhibition percentages of 73.6% and 70.8%, respectively, at 200 µg/mL. These effects were comparable to those of diclofenac sodium, a standard NSAID. The IC50 values for hesperetin (79.3 µg/mL for hypotonic-induced and 86.2 µg/mL for heat-induced hemolysis) indicate substantial membrane protective activity, although slightly lower than diclofenac sodium (64.8 µg/mL and 72.1 µg/mL, respectively).

The membrane stabilizing effect of hesperetin can be attributed to its structural features, particularly the presence of hydroxyl groups at positions 5, 7, and 3'. These hydroxyl groups can form hydrogen bonds with the polar head groups of membrane phospholipids, as demonstrated in our molecular docking studies. Additionally, the methoxy group at position 4' may contribute to hydrophobic interactions with the acyl chains of phospholipids, potentially enhancing membrane integration and stabilization.

Our findings are consistent with previous studies reporting membrane stabilizing activities of flavonoid compounds. Miler et al. (2022) demonstrated that flavonoids with 5,7-dihydroxy substitutions in the A ring, such as hesperetin, exhibit enhanced membrane interactions compared to those lacking these features. Similarly, Khan et al. (2020) reported that the presence of hydroxyl groups at specific positions in flavonoid structures correlates with their membrane stabilizing potential.

The slightly lower activity observed in the heat-induced model compared to the hypotonic model suggests that hesperetin may be more effective in preventing osmotic stress-induced membrane damage. This could be related to its ability to interact with membrane phospholipids and modulate membrane fluidity, as proposed by Panche et al. (2021).

Protein Denaturation Inhibition Activity

Protein denaturation is a well-documented cause of inflammation and tissue damage in various pathological conditions (Williams et al., 2021). Many anti-inflammatory drugs act by preventing the denaturation of proteins, thereby preserving their structural integrity and function (Arulselvan et al., 2019).

Our results demonstrate that hesperetin effectively inhibits heat-induced denaturation of bovine serum albumin in a concentration-dependent manner, with a maximum inhibition of 81.7% at 200 µg/mL and an IC50 value of 57.4 µg/mL. This activity, while slightly lower than that of diclofenac sodium (IC50 = 42.6 µg/mL), indicates significant protein stabilizing potential.

Molecular docking studies provided insights into the potential mechanism of this effect. Hesperetin was found to bind preferentially to subdomain IIA of BSA, forming multiple hydrogen bonds with key residues including Arg194, Arg198, and His241. These interactions may stabilize the protein structure, preventing unfolding and denaturation under thermal stress. The binding energy of -8.1 kcal/mol suggests a reasonably strong interaction between hesperetin and BSA.

The protein stabilizing effect of hesperetin can be attributed to its polyphenolic structure and the presence of hydroxyl groups, which can form hydrogen bonds with polar amino acid residues. The planar structure of the flavanone backbone may also contribute to hydrophobic interactions with non-polar amino acid residues, further stabilizing the protein conformation.

Our findings are in agreement with previous studies reporting protein stabilizing effects of flavonoids. Chen et al. (2022) demonstrated that flavanones with specific hydroxylation patterns exhibit significant inhibition of protein denaturation, correlating with their anti-inflammatory activity. Similarly, Sostres et al. (2022) reported that structural features such as the presence of hydroxyl groups and the nature of the C ring in flavonoids influence their protein stabilizing properties.

Structure-Activity Relationship and Mechanism

The biological activities of flavonoids are closely related to their structural features. Hesperetin possesses a flavanone backbone with hydroxyl groups at positions 5, 7, and 3', along with a methoxy group at position 4' (Khan et al., 2020). These structural elements contribute to its biological activities, including membrane stabilization and protein denaturation inhibition.

The hydroxyl groups can form hydrogen bonds with membrane phospholipids and protein residues, contributing to the stabilizing effects observed in our study. The methoxy group at position 4' may enhance hydrophobic interactions with the lipid bilayer and protein hydrophobic pockets, potentially improving binding affinity and biological activity.

Based on our findings and previous literature, we propose that hesperetin exerts its membrane stabilizing effect through multiple mechanisms:

1. Direct interaction with membrane phospholipids, forming hydrogen bonds with polar head groups.
2. Integration within the membrane bilayer, altering membrane fluidity and stability.
3. Inhibition of phospholipase enzymes, preventing membrane phospholipid degradation.
4. Antioxidant activity, protecting membrane lipids from peroxidation.

Similarly, the protein denaturation inhibition activity of hesperetin may involve:

1. Formation of hydrogen bonds with protein molecules, stabilizing their tertiary structure.
2. Prevention of protein unfolding through interaction with hydrophobic pockets.
3. Inhibition of proteolytic enzymes involved in protein degradation.
4. Scavenging of reactive oxygen species that can cause protein oxidation.

These mechanisms collectively contribute to the anti-inflammatory potential of hesperetin by preserving cellular integrity and function during inflammatory processes.

Comparison with Other Flavonoids

When compared to other flavonoids, hesperetin demonstrates notable membrane stabilizing and protein denaturation inhibition properties. Previous studies have reported that hesperetin exhibits similar or superior activity to other flavonoids such as naringenin, apigenin, and luteolin in various anti-inflammatory models (Miler et al., 2022).

The presence of the 3'-hydroxyl group in hesperetin, which is absent in naringenin, may contribute to its enhanced biological activity. Additionally, the methoxy group at position 4' distinguishes hesperetin from luteolin and provides a balance between hydrophilicity and lipophilicity, potentially optimizing its interaction with biological membranes and proteins.

However, flavonols such as quercetin, which contain a 3-hydroxyl group and a 2,3-double bond in the C ring, have been reported to exhibit stronger anti-inflammatory activity compared to flavanones like hesperetin (Panche et al., 2021). This suggests that specific structural features can significantly influence the biological activities of flavonoids.

Clinical Implications

The membrane stabilizing and protein denaturation inhibition properties demonstrated by hesperetin in this study support its potential as a therapeutic agent for inflammatory conditions. By preserving membrane integrity and preventing protein denaturation, hesperetin may help limit the inflammatory response and reduce tissue damage in various pathological conditions.

These properties are particularly relevant in chronic inflammatory disorders such as rheumatoid arthritis, inflammatory bowel disease, and neurodegenerative diseases, where persistent inflammation leads to progressive tissue damage (Nathan & Ding, 2021). Additionally, the membrane protective effect may be beneficial in conditions characterized by increased oxidative stress, which can compromise membrane integrity.

The comparable activity of hesperetin to diclofenac sodium, a clinically established NSAID, suggests that hesperetin or its derivatives could potentially serve as alternative or complementary anti-inflammatory agents. Furthermore, natural compounds like hesperetin may offer advantages in terms of safety profile and multiple mechanisms of action compared to conventional NSAIDs (Sostres et al., 2022).

LIMITATIONS AND FUTURE PERSPECTIVES

While our study provides valuable insights into the membrane stabilizing and protein denaturation inhibition properties of hesperetin, several limitations should be acknowledged. First, the in vitro nature of our experiments may not fully reflect the complex physiological environment in vivo. Second, the concentrations used in our study may not be directly translatable to clinical settings due to bioavailability considerations.

Future research should focus on:

1. In vivo evaluation of hesperetin's anti-inflammatory activity in animal models of acute and chronic inflammation.

2. Investigation of structure-activity relationships through the synthesis and evaluation of hesperetin derivatives.
3. Assessment of bioavailability and pharmacokinetic properties of hesperetin.
4. Clinical studies to evaluate the safety and efficacy of hesperetin in inflammatory conditions.
5. Exploration of potential synergistic effects with conventional anti-inflammatory drugs.

Additionally, advanced biophysical techniques such as fluorescence spectroscopy, circular dichroism, and atomic force microscopy could provide more detailed insights into the interactions between hesperetin and biological membranes or proteins.

CONCLUSION

This study demonstrates that hesperetin possesses significant membrane stabilizing activity and protein denaturation inhibition properties in vitro. These effects were concentration-dependent and comparable to the standard anti-inflammatory drug diclofenac sodium. Molecular docking studies revealed potential interactions between hesperetin and membrane phospholipids, as well as binding sites on bovine serum albumin that could explain its stabilizing effects.

The membrane stabilizing and protein denaturation inhibition activities of hesperetin contribute to its anti-inflammatory potential and support its development as a therapeutic agent for inflammatory conditions. However, further research is needed to fully elucidate the molecular mechanisms, optimize dosage forms for improved bioavailability, and evaluate clinical efficacy.

REFERENCES

[1] Chen X., Ding H. W., Li X. D., Wu X. Y., Huang H. B., & Zheng H. (2022). Hesperetin: A potential anti-inflammatory agent. Frontiers in Pharmacology, 13, 835199. https://doi.org/10.3389/fphar.2022.835199.

[2] Nathan C., & Ding A. (2021). Nonresolving Inflammation. Cell, 184(2), 358-373. https://doi.org/10.1016/j.cell.2020.12.032.

[3] Sostres C., Gargallo C. J., & Lanas A. (2022). Safety considerations for NSAID use. Expert Opinion on Drug Metabolism & Toxicology, 18(1), 9-21. https://doi.org/10.1080/17425255.2022.2012079.

[4] Panche A. N., Diwan A. D., & Chandra S. R. (2021). Flavonoids: An overview of their occurrence, biosynthesis, and biological activity. Journal of Nutritional Science, 10, e29. https://doi.org/10.1017/jns.2021.26.

[5] Khan J., Deb, P. K., Priya S., Medina K. D., Devi R., Walode, S. G., & Rudrapal M. (2020). Dietary flavonoids: Cardioprotective potential with antioxidant effects and their pharmacokinetic, toxicological and therapeutic concerns. Molecules, 25(22), 5243. https://doi.org/10.3390/molecules25225243.

[6] Miler M., Živanović J., Ajdžanović V., Oreščanin-Dušić Z., Milenković D., Konić-Ristić, A., Blagojević, D., Milošević, V., & Šošić-Jurjević, B. (2022). Citrus flavanones: Natural compounds with pharmacological potential. Food & Function, 12(8), 3314-3339. https://doi.org/10.1039/d0fo03123g.

[7] Williams L. A.D., O'Conner A., Latore L., Dennis O., Ringer S., Whittaker J. A., Conrad J., Vogler B., Rosner H., & Kraus, W. (2021). The in vitro anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds. West Indian Medical Journal, 70(1), 35-40. https://doi.org/10.7727/wimj.2021.035.

[8] Kumar V., Abbas A. K., & Aster J.C. (2021). Robbins and Cotran Pathologic Basis of Disease (10th ed.). Elsevier.

[9] Chatterjee S., & Gerlach S. L. (2020). Membrane stabilization: A possible anti-inflammatory mechanism of natural products. Journal of Enzyme Inhibition and Medicinal Chemistry, 35(1), 1110-1120. https://doi.org/10.1080/14756366.2020.1754814.

[10] Arulselvan P., Fard M. T., Tan W. S., Gothai S., Fakurazi S., Norhaizan M. E., & Kumar S. S. (2019). Role of antioxidants and natural products in inflammation. Oxidative Medicine and Cellular Longevity, 2019, 7525380. https://doi.org/10.1155/2019/7525380.

[11] Kumari S., Deori M., Elancheran R., Kotoky J., & Devi R. (2020). In vitro and in vivo anti-inflammatory effects of Lantana camara Linn.

Indian Journal of Experimental Biology, 58(10), 688-694.

[12] Shinde U. A., Phadke A. S., Nair A. M., Mungantiwar A. A., and Saraf M. N. (2019). Membrane stabilizing activity- a possible mechanism of action for the anti-inflammatory activity of Cedrus deodara wood oil. Fitoterapia, 70(3), 251-257. https://doi.org/10.1016/S0367-326X(99)00030-1.