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Anti-Inflammatory Activity of Purified 36.3 kDa Coleus Aromatics Leaves Protein: An in Vitro Study

Santhosh Kumar Nune [a], Alia Nusruth [b], Ramesh Babu Elle [c], Dinesha Ramadas [d, *]

[a] *Department of Biochemistry, Prathima Relief Institute of Medical Sciences, Hanamkonda, Telangana, India*
[b] *Department of Biochemistry, Adichunchanagiri Institute of Medical Sciences, Adichunchanagiri University, Karnataka, India*
[c] *Department of Anatomy, Shridevi Institutes of Medical Sciences & Research Hospital, Tumkur, Karnataka, India*
[d] *Department of Biochemistry, Adichunchanagiri School of Natural Sciences, Adichunchanagiri University, Karnataka, India*

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ABSTRACT

Background and aim: Medicinal plants have recently become the focus of intense study for conservation, as documented pharmacological effects support their traditional uses. This study aims to investigate the anti-inflammatory activity of purified 36.3 kDa Coleus aromaticus leaves protein (CALP) using the Human Red Blood Cells (HRBC) membrane stabilization method. Purified 36.3kDa CALP is used for this study.

Material and methods: The HRBC membrane stabilization method was employed to investigate the anti-inflammatory activity of CALP. All data were expressed as mean ± standard deviation of three replicates (n=3).

Results: The anti-inflammatory activity of the purified CALP was concentration-dependent; as the concentration increased, the percentage of protection also increased. The purified CALP shows a maximum anti-inflammatory activity of 65.93% (±3.12) at 500 µg/ml, which is slightly lower than that of the standard drug Diclofenac sodium [80.34% (±3.90)] at 200 µg/ml. The purified Coleus aromaticus leaves protein exhibits a membrane stabilization effect by inhibiting hypotonicity-induced lysis of erythrocyte membrane.

Conclusions: This study concludes that the purified 36.6 kDa CALP exhibits a stronger membrane-stabilizing property than the standard drug Diclofenac sodium and thus has stronger anti-inflammatory activity. Proteins will continue to serve as a reservoir for the development of potent drugs with less severe and life-threatening adverse effects; however, these results need to be confirmed by in vivo anti-inflammatory studies.

1. Introduction

Inflammation is a normal host defense response to tissue damage caused by infection or injury, involving the infiltration of monocytes, dendritic cells, and neutrophils.[1] The pathogens provoke an acute inflammatory response that results in the removal of irritants; however, inadequate resolution with prolonged inflammatory response leads to chronic inflammation, predisposing to various diseases, including cancer.[2] The acute inflammatory response begins with the neutrophils, which initially destroy foreign particles and remove the damaged tissue. As inflammation continues, macrophages appear at the site of inflammation and initiate phagocytosis.[3] This phagocytosis plays a crucial role in inflammatory diseases through the secretion of reactive oxygen species, such as hydrogen peroxide, and reactive nitrogen species, including nitric oxide, as well as a range of other inflammatory mediators, including prostaglandins.[4] There are many different types of drugs with relevant clinical applications for treating inflammatory disorders. These drugs are not only costly but are also associated with undesirable side effects in some patients. Functional foods are natural products that have been found to have a beneficial effect on modulating inflammatory responses by interacting with many inflammatory mediators. Hence, natural products may be a valuable source for identifying bioactive compounds and potentially developing new drugs.[5] Among these compounds, proteins and bioactive peptides derived from animal and plant sources are the most extensively studied components. Currently, there is a growing trend in the use of food protein-derived peptides as intervention agents against chronic diseases.[6-7] Coleus aromaticus Benth., (Lamiaceae), syn. C. ambonicus (Lour.) Spreng. or Plectranthus ambonicus (Lour.), is known as Indian/country borage. Coleus aromaticus (Karpurvalli) is a household herb grown in kitchen gardens and is used for culinary purposes.[8] The majority of studies have focused on the phytochemical profiles of alkaloids, polyphenols, and flavonoids, as well as pharmacognostic

* *Corresponding author. Dinesha Ramadas*
E-mail address: *drdinesha@acu.ac.in*
Department of Biochemistry, Adichunchanagiri School of Natural Sciences, Adichunchanagiri University, Karnataka, India
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investigations. They act as antidiabetics, anti-inflammatories, antioxidants, antibacterials, anti-microbiological, hepatoprotectives, anticarcinogens, and anti-mutagens.[9-19] In vitro studies have shown that Coleus aromaticus exhibits significant anti-inflammatory activity due to its bioactive components and has been investigated for potential pharmaceutical drug formulations. However, the role of the protein portion of Coleus aromaticus leaves as an anti-inflammatory activity has not been studied to date. Hence, the present study investigated the anti-inflammatory activity of the purified protein from Coleus aromaticus leaves, providing scientific evidence for its traditional use.

2. Material and Methods

All chemicals and reagents used were of analytical grade, and the anti-inflammatory activity was performed according to standard methods. Coleus aromaticus protein was separated from other phytochemical constituents and purified by using Sephadex G-50 Column Chromatography, SDS-PAGE, HPLC, and MALDI-MS. The purified protein portion of Coleus aromaticus leaves had shown significant antioxidant activity, a protective role against t-BOOH- and H2O2-induced DNA damage, and a protective effect on the cell viability of human peripheral lymphocytes against damage induced by t-BOOH& H2O2, which was reported earlier in previous progress reports. The experimental protocol was approved by the Institutional Animal Ethical Committee (CPCSEA Protocol approval No.AIMS/PhD/404/16-17). The experiment was conducted in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Government of India. In this study, purified Coleus aromatic leaves protein (CALP) was investigated to analyze its anti-inflammatory activity using the Human Red Blood Cells (HRBC) membrane stabilization method. The concentrations of the solvents and the effective concentrations of standards were used based on the literature of Chikkanna.

In vitro anti-inflammatory activity

Membrane stabilization assay

The Human Red Blood Cell (HRBC) membrane stabilization method was used to study the anti-inflammatory activity of CALP.[13] Blood was collected from healthy donors and mixed with an equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm, and the packed cells were washed with isotonic saline (0.85%, pH 7.2). A suspension was then made with isotonic saline (10% v/v) (HRBC suspension).

The assay mixture contained 1 mL of Phosphate buffer (0.15 M, pH 7.4), 2mL of hyposaline (0.36%), 0.5 mL of HRBC suspension, and 1 mL of various concentrations of the purified CALP. Diclofenac sodium was used as the standard drug. In the control solution, instead of hyposaline, 2ml of distilled water was added. The mixtures were incubated at 37 °C for 30 min and centrifuged. The absorbance of the supernatant solution was measured spectrophotometrically at 560nm. The % haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization was calculated using the formula.

$$\%\text{Membrane stabilization} = 100 - \left(\frac{\text{OD of Test sample}}{\text{O.D. of Control}} \text{ X100}\right)$$

Statistical analysis

All data were expressed as mean ± standard deviation of three replicates (n=3) with a Student's t-test.

3. Results

The anti-inflammatory activity of the purified CALP was concentration-dependent, with an increase in concentration leading to an increase in % protection. (Table 1) The purified CALP shows a maximum of 65.93% (±3.12) anti-inflammatory activity at a concentration of 500µg/ml, which is slightly lower than that of the standard drug Diclofenac Sodium [80.34% (±3.90)] at a concentration of 200µg/ml. The proteins may exhibit a membrane stabilization effect by inhibiting the hypotonicity-induced lysis of the erythrocyte membrane. The erythrocyte membrane is comparable to the lysosomal membrane; hence, its stabilization suggests that the purified CALP may also stabilize the lysosomal membrane. (Fig. 1)

Table 1. Absorbance (at 660nm) and % membrane stabilization of the purified CALP & Diclofenac sodium in different concentrations.

Concentration (µg/ml)	Purified *CALP*		Diclofenac sodium*	
	Absorbance	% Protection	Absorbance	% Protection
100	0.309± 0.06	24.99 ± 1.51	0.185 ± 0.02	54.69 ± 6.44
200	0.276± 0.01	32.82 ± 3.42	0.081 ± 0.01	80.34 ± 3.90
300	0.235 ± 0.03	42.71± 2.91	0.089 ± 0.07	78.39 ± 1.74
400	0.190 ± 0.01	53.88 ± 0.23	0.087 ± 0.01	78.71 ± 2.68
500	0.140± 0.01	65.93 ±3.12	0.098 ± 0.06	76.21 ± 1.47
600	0.143± 0.03	65.12± 0.60	0.097 ± 0.05	76.29 ± 1.40

Date represented as Mean ±SD (n=3), *Standard drug and Control is 0.421.

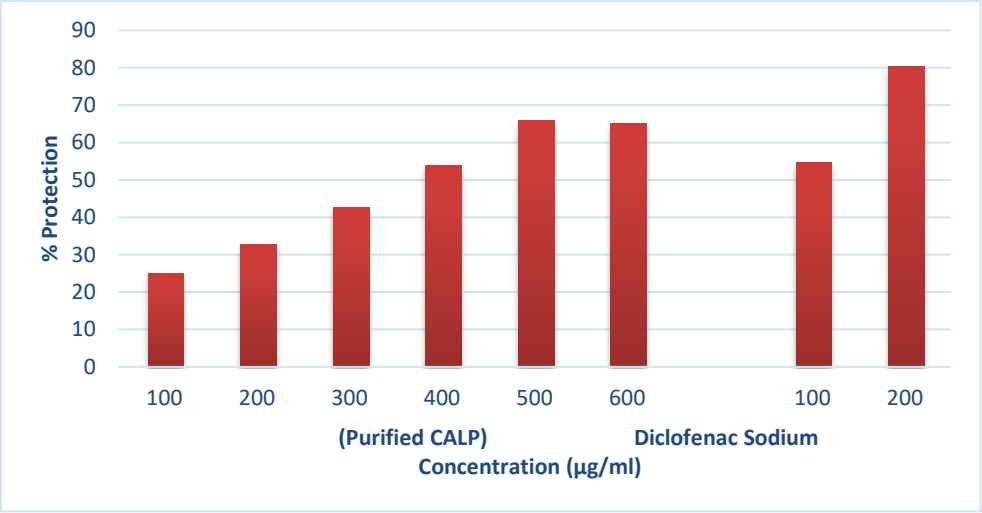

Fig. 1. In-vitro anti-inflammatory effect of purified *Coleus aromaticus* leaves Protein (CALP).

4. Discussion

Inflammation is a defense response of the body to hazardous stimuli, such as allergens or tissue injury. However, an uncontrolled inflammatory response causes chronic inflammatory disorders. Due to the significant side effects of steroidal and NSAID medications, which are commonly used as anti-inflammatory agents in practice, there is a growing interest in natural compounds, such as dietary supplements and herbal remedies, that have been used for centuries to reduce pain and inflammation. Thus, we need to incorporate natural anti-inflammatory factors into medication therapy to achieve a more pronounced pharmacological response and minimize unwanted side effects.[10] Various study results showed that bioactive compounds act through different mechanisms to prevent and suppress inflammatory responses. Neelam Begumetal., in 2009, demonstrated the anti-inflammatory activity of the aqueous extract of Coleus Aromaticus by inhibiting the release of mediators of inflammation.[11] Similarly, a study by Nirmala Devi. N et al. (2010) studied the ethanolic and water extracts of P. Amboinicus. They reported that the ethanolic extract had higher membrane stabilization properties, which were comparable to those of the standard drug Sodium Hydrocortisone.[12] Chiu YJ et al. (2012) suggest that P. Amboinicus has a role in relieving pain and inflammation by inhibiting pro-inflammatory mediators through the blockade of NF-κB activation.[13] Janakiraman et al., (2014) showed that the hydroalcoholic extract of leaves from P. Amboinicus possesses a significant anti-inflammatory effect against in vitro protein denaturation, due to the presence of polyphenolic content, and may have a synergistic effect rather than the activity of a single compound.[14] A similar study on the anti-inflammatory effect of proteins extracted from natural sources was reported by Chan-Zapata et al., in 2019. They demonstrated that a protein model isolated from Salvia hispanica L. seeds showed a significant anti-inflammatory effect without altering the viability of macrophages. Furthermore, this protein had a repressive effect on ear edema, as well as an inhibitory effect on the DNFB-induced type hypersensitivity model.[15] Earlier researchers at our laboratory (AIMS-CRL, Adichunchanagiri Institute for Molecular Medicine) have reported the presence of potent proteins with excellent anti-inflammatory and antioxidant properties in in vitro systems, in various plant extracts, including Pippali (Piper longum) [16] and Zingiber officinale root.[17] In this study, the results show that the purified CALP also exhibits significant anti-inflammatory activity in a concentration-dependent manner, which is comparable to that of the standard drug Diclofenac sodium.

5. Conclusion

This study concludes that the purified 36.3 kDa CALP exhibits a significant membrane stabilization property compared to the standard drug Diclofenac sodium, and thus has significant anti-inflammatory activity. Proteins will continue to serve as a reservoir for the development of potent drugs with less serious and life-threatening adverse effects; however, these results need to be confirmed by in vivo anti-inflammatory studies.

Conflict of Interest

The authors declared that there is no conflict of interest.

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