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Review

Egg albumin denaturation assay for *in vitro* evaluation of anti-inflammatory activity of *Caesalpinia crista* seed

Anupriya Thomas*, Rifa Fathima. NA, Eldhomon. PR, Keerthana. S, Sharika Jebin. M

*Associate Professor, Department of Pharmacology, Ahalia School of Pharmacy, Kozhippara, Pudussery East, Kerala 678557

*Corresponding Author: Anupriya Thomas
E.mail Id: preldhomon@gmail.com

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Abstract

Protein denaturation, one of the intricate biological processes involved in inflammation, leads to tissue damage and inflammatory diseases. Using the egg albumin protein denaturation assay, the current study assessed the ethanolic seed extract of Caesalpinia crista's *in vitro* anti-inflammatory effectiveness. Following Soxhlet extraction of the seeds, flavonoids, alkaloids, tannins, saponins, and other secondary metabolites were detected by first phytochemical screening. Indomethacin was utilized as the reference medication, and anti-inflammatory activity was evaluated at several doses (20–100 µg/mL). With a maximal inhibition of 90.47%, the ethanolic extract demonstrated concentration-dependent inhibition of protein denaturation, demonstrating notable efficacy at higher doses. The extract showed noteworthy protein stabilizing characteristics, while having a somewhat lower activity than indomethacin. The presence of bioactive phytoconstituents may be responsible for the effect that has been seen. These results imply that *Caesalpinia crista* seeds have encouraging anti-inflammatory properties and merit additional mechanistic and in vivo research.

Keywords: Protein denaturation, Indomethacin, *Caesalpinia crista*

1.INTRODUCTION

Caesalpinia crista is a medicinal herb of family *Caesalpiniaceae* found in subtropical and tropical regions of Southeast Asia.[1]

Inflammation is a complicated process that often causes pain. It includes the rise of vascular permeability, the increase of protein denaturation, and the change of membranes.

Protein denaturation is the process by which proteins lose their secondary and tertiary structures due to external pressures or chemicals such as heat, an organic solvent, a concentrated inorganic salt, or a strong acid or base. When proteins are denatured, their biological functions are lost. One of the main characteristics of inflammation is protein denaturation.[4]

Thus, a medication's or a therapeutic product's capacity to prevent or lessen protein denaturation may have the potential to prevent or manage inflammation.[5] As protein denaturation inhibition tests, heat-induced egg albumin and bovine serum albumin denaturation inhibition tests are commonly employed.[6]

Protein denatures and makes the reaction mixture more turbid when heated. This leads to an increase in the absorbance of the mixture. If a potential drug/plant product has anti-inflammatory properties, it will inhibit protein denaturation, lowering the progressive increase in absorbance due to protein denaturation. [2]

The primary objective of the egg albumin denaturation assay is to evaluate whether agents or compounds can inhibit or prevent the denaturation of egg albumin under specific conditions. Egg albumin serves as a model protein in the experiment, with denaturation induced by exposure to extreme heat, pH, or other denaturing agents.

Denaturation disrupts the native conformation of egg albumin, altering its physical properties and abolishing its functional activity. The assay evaluates a drug or compound's ability to prevent or reduce denaturation, thereby assessing its potential anti-inflammatory effects. This approach is based on the principle that anti-inflammatory agents can stabilize protein structures and inhibit denaturation a process often associated with inflammation and tissue damage. Consequently, compounds that significantly reduce egg albumin denaturation may possess anti-inflammatory properties. Protein denaturation is considered one cause of inflammation, and non-steroidal anti-inflammatory drugs (NSAIDs) simultaneously inhibit protein denaturation and COX enzyme activity.[3]

2.METHOD

2.1 Chemicals. Egg albumin, Phosphate buffered saline, Dimethyl Sulfoxide (DMSO), Disodium hydrogen Phosphate (Na2HPO4), Potassium dihydrogen phosphate (KH2PO4), Sodium Chloride, Distilled water, Indomethacin.

2.2 Plant Material. The Seeds of *C. crista* were collected from the Wayanad, Kerala India. It was identified, authenticated, and Certified from Dr. S.S. Hameed, Department of Botany, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

2.3 Sample Preparation. Dried seeds of *Caesalpinia crista* were used. The seed coat was manually broken, and the Testa was separated. The Seeds were powdered and passed through sieve No. 40. The powder was stored in an airtight container. Place the powdered material in a thimble and insert it into the main chamber of the Soxhlet apparatus. Pour the ethanol in a round bottom flask (RBF). Heat the solvent to its boiling point and carry out the extraction. The powder was defatted with petroleum ether in a Soxhlet apparatus for 24 hours. After defatting, the powder was dried and recharged into the same Soxhlet extractor. It was then extracted with ethanol for 16 hours. The ethanolic extract yielded a reddish-brown sticky mass.

2.4 In Vitro method using Egg Albumin Protein Denaturation.

2.4.1 Preparation of Reagents.

Phosphate Buffered Saline (ph. 7.4); Dissolve 2.38g of disodium hydrogen phosphate, 0.19g of potassium dihydrogen phosphate and 8.0g of sodium chloride in

sufficient water to produce 1000ml. adjust the pH if necessary.[7]

Test solution: it consists of 0.2ml of Egg albumin and 0.05ml of test solution of various concentrations.

Test control solution: it consists of 0.2ml of Egg albumin and 0.05ml of dimethyl sulfoxide (Solvent).

Standard solution: it consists of 0.2ml of Egg albumin and 0.05ml of indomethacin (Standard drug) of various concentrations.

2.4.2 Procedure

A reaction mixture is prepared by adding 0.2ml egg albumin (fresh hen's egg) and 2.8ml phosphate buffer solution (ph.7,4) in a clean beaker.To this prepared solution, 2 mL of the test extract at varying concentrations (20, 40, 60, 80, and 100 µg/mL) was added into separate test tubes. A control solution was also prepared using distilled water instead of the test extract. All the reaction mixtures were adjusted to the required volume and mixed thoroughly.

The resulting reaction mixture was incubated at37ºC for 15 minutes to allow interaction between the protein and the test extract. and afterwards heated to 70ºC for 5minutes.After heating, the samples were allowed to cool to room temperature. The absorbance of each sample was measured at 660 nm using a UV–Visible spectrophotometer. The percentage inhibition of protein denaturation was calculated by comparing the absorbance of the test samples with that of the control.

3. RESULTS

3.1 Calculation of percentage yield of extracts

Percentage yield (% w/w) was calculated for extracts.

Percentage yield=weight in gram of extract obtained/weight in gram of plant material taken × 100

The percentage yield obtained is **9.28%w/w.**

3.2 Preliminary phytochemical screening.

Preliminary phytochemical screening of the ethanolic seed extract of *Caesalpinia crista* was carried out to find the various phytoconstituents. This preliminary screening indicated the presence of major secondary metabolites including carbohydrate, flavonoids, alkaloids, tannins, saponins, coumarin glycosides, reducing sugars, triterpenoids and fatty acids and proteins.

Phytochemicals	Result
Carbohydrate	+
Alkaloids	+
Flavonoids	+
Saponin	+
Proteins and Amino acids	+
Tannins	+
Coumarin glycoside	+

\+ Color produced

3.3 Egg albumin- induced protein denaturation assay

	CONCENTRATION	ABSORBANCE	TEST CONTROL	% INHIBITION
TEST (Ethanolic extract)	**20**	**0.023**	**0.021**	**9.52**
	40	**0.026**		**23.80**
	60	**0.032**		**52.30**
	80	**0.038**		**80.95**

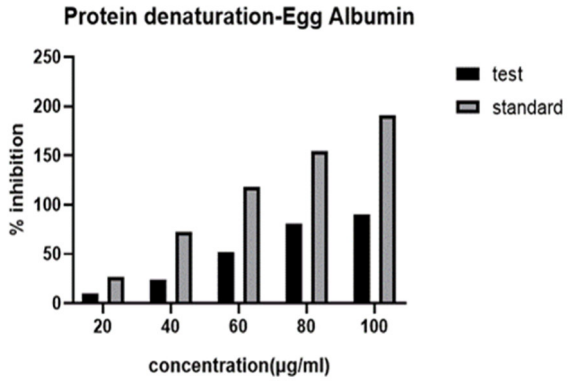
	100	**0.040**		**90.47**
STANDARD (Indomethacin)	**20**	**0.014**	**0.011**	**27.27**
	40	**0.019**		**72.72**
	60	**0.024**		**118.18**
	80	**0.028**		**154.54**
	100	**0.032**		**190.90**

The graph shows the test extract's and the standard medication's (indomethacin) in vitro anti-inflammatory activity using the egg albumin protein denaturation assay. From 20 to 100µg/mL, both showed concentration-dependent increases in percentage inhibition.

The standard (indomethacin) showed somewhat higher inhibition, from 27% to 190%, whereas the test extract showed an increase from about 9% to 90%. Significant anti-inflammatory potential was indicated by the extract's activity, which at higher concentrations was almost equal to that of indomethacin.

These findings support the extract's potential as an anti-inflammatory agent by showing that it successfully prevents protein denaturation, most likely as a result of phytochemicals like flavonoids and phenolics.

3.4 Graphical representation.



The *in vitro* anti-inflammatory activity of the ethanolic extract was evaluated using the protein denaturation assay with egg albumin, and the outcomes were contrasted with those of the common medication indomethacin. Because egg albumin is similar to physiological proteins in humans and is highly sensitive to heat-induced denaturation, it was selected. At a maximum of 90.4%, the ethanolic extract showed concentration-dependent inhibition of protein denaturation, with little activity at 100 µg/mL and noticeable inhibition at 800–1000 µg/mL. The activity of indomethacin was similar, with a maximum inhibition of 190.9%. The extract's overall performance was comparable to that of the standard, suggesting that it has promising anti-inflammatory potential.

3.5 IC_{50} Value

The IC_{50} value of the egg albumin denaturation assay was found to be 59 µg/mL for the test sample, whereas the standard drug exhibited a lower IC_{50} value of 31 µg/mL, indicating comparatively higher anti-inflammatory activity of the standard.

4.CONCLUSION

Caesalpinia crista's ethanolic seed extract demonstrated strong anti-inflammatory and anti-arthritic properties in vitro by efficiently preventing egg albumin-induced protein denaturation in a concentration-dependent manner. Despite having a weaker inhibitory effect than indomethacin, the extract exhibited similar activity at higher concentrations, suggesting a significant potential for protein stabilization. It is probable that phytoconstituents like flavonoids and phenolic compounds are responsible for the activity. Further *in vivo* and mechanistic research is necessary in light of these findings, which support the therapeutic relevance of *C. crista* seeds as a possible natural source of anti-inflammatory agents.

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