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In Vitro Anti Inflammatory and Anti Arthritic Activity of *Commelina benghalensis L.* Leaves

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ABSTRACT

Introduction

Commelina benghalensis L. commonly known as **Benghal dayflower,** belongs to the family Commelinaceae. It is widely used for the treatment of wounds and skin diseases.

Aim

The current study focuses on the evaluation of in vitro anti-inflammatory and antiarthritic property of the leaf extracts of*Commelina benghalensis L.*

Methods

The hydroalcoholic extract (70%) of *Commelina benghalensis L.* (Leaf) was subjected to anti-inflammatory and anti arthritic activity by membrane stabilisation and inhibition of protein denaturation method were determined.

Results

The inhibitory concentration (IC_{50}) of HAECB in HRBC membrane stabilization study was found to be 69µg/ml in comparison with diclofenac sodium 57µg/ml. It showed moderate anti-inflammatory activity. The inhibitory concentration (IC_{50}) of HAECB in protein denaturation was found to be 17µg/ml in comparison with diclofenac sodium 14µg/ml. It showed moderate anti-arthritic activity.

Conclusion

HAECB showed moderate anti-inflammatory activity which may be due to the strong occurrence of polyphenolic compounds such as flavonoids, tannins and phenols. HAECB has shown moderate anti-arthritic activity which may be due to the phenolic constituent.

Keywords: Anti-inflammatory, Anti-arthritic, *Commelina benghalensis L.*

INTRODUCTION

Medicinal and culinary herbs are rich sources of anti-inflammatory compounds such as flavonoids. Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling

and pain. Prolonged inflammation leads to the rheumatoid arthritis, atherosclerosis, hey fever, ischemic heart diseases and inflammation is a common manifestation of infectious diseases like leprosy, tuberculosis, syphilis, asthma, inflammatory bowel syndrome, nephritis, vascularitis, celiac diseases, auto-immune diseases etc. Anti-inflammatory drugs like NSAIDs used to reduce the swelling and pain of inflammation. But these agents carry the risk of gastro-intestinal toxicity, cardiovascular and other toxicity for prolonged use. For these reason, there is a need for ant-inflammatory drugs having less severe side effects to use for chronic inflammatory disease as well. Therefore, in recent time, more interest is shown in alternative and natural drugs for treatment of various diseases, but there is a lack of proper scientific evidence [1].

Leukocytes, the key players of inflammatory response, can eliminate microbes and dead cells by phagocytosis, followed by their destruction in phagolysosomes. Destruction is caused by free radicals generated in activated leukocytes (neutrophils and monocytes) and lysosomal enzymes. Enzymes and reactive oxygen species may be released into the extracellular environment where it acts as mediators of inflammation. Such mediators are mainly arachidonic acid metabolites, generated through Cyclooxygenase and Lipoxygenase pathways. Most of the anti-inflammatory drugs are targeted on these pathways.

In a different approach, rather than blocking a particular mediator or its pathway, preventing the release of inflammatory mediators could be considered as a better option. The possibility of this approach is revealed in this research by studying the ability of the plant extract to prevent the lysosomal membrane destruction. An effective way to study this activity in vitro is to study the HRBC membrane stabilization activity of the plant extract. Lysosomal membrane and RBC membrane are similar in structure apart from the fact that luminal surface of the lysosomal membrane contains a glycoprotein coat which protects the membrane from digestion by lysosomal acid hydrolases. This method has been used in most preliminary anti-inflammatory screening procedures [2].

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint swelling, synovial inflammation and cartilage destruction and commonly lead to significant disability. According to WHO, 0.3-1% of the world population is affected from rheumatoid arthritis (RA) and among them females are three times more prone to the diseases compared to males. It caused by no of proinflammatory molecules released by macrophages including reactive oxygen species and ecosanoids such as prostaglandins, leukotrines and cytokines. The regulation of these mediators secreted by macrophages and other immune cells and modulation of arachidonic acid metabolism by inhibiting enzymes like cox and lox are the potential target for chronic inflammatory conditions. Eventhough various categories like immunosuppressants, NSAIDs, steroidal anti-inflammatory drugs are being used till now, the potential side effects give a limitation for their use.Now it is a growing concernal lover for the development of new safe, potent, less toxic antiarthritic drug. Hence, there is a need to explore for more naturally available alternatives, so that their therapeutic values can be assessed and expanded [3].

Commelina benghalensis L. commonly known as **Benghal day flower,** belongs to the family Commelinaceae. *Commelina benghalensis L.* is a perennial herb native to tropical Asia and Africa.

Valaiyans of Piranmalai hills, Tamilnadu used the leaves for the treatment of rabies and wounds [4&5]. Bangladesh the kavirajes tribals used the young leaves for external poisoning [6].

The phytochemical screening of previous studies of *Commelina benghalensis L* revealed the presence of tannins, phlobatannins, saponins, flavonoids, alkaloids, steroids and flavonoids, carbohydrates, phytosterol, terpenoids, quinon, volatile oil, anthraquinone [7-15]. GC-MS analysis of *Commelina benghalensis L.* revealed the presence of bioactive compounds such as 3-dodecene, 1-hexadeconol, 9-eicosene and tetratriacontane, Phenol 2,4 bis(1,1 dimethyl ethyl), hexadecen1 ol trans9, 9,10 anthracenedione, tetracosane, 1,4 benzene-dicarboxylic acid, bis (2ethylhexyl) ester, 13 docosenamide, tetracosane 11 decyl [16].

The plant exhibited various pharmacological activites such as anti inflammatory activity [9], 15-lipooxygenase inhibition, anticoagulant activity [13&14], antibacterial activity [15,19&20], antimicrobial activity [17&18], antiplasmodial activity, thrombolytic and cytotoxic activity and antidiarrhoeal & anthelmintic activity [21-23].

The current study focuses on the evaluation of in vitro anti-inflammatory and antiarthritic property of the leaf extracts of *Commelina benghalensis L.*

MATERIALS AND METHODS

Plant Collection & Authentication

Fresh leaf of *Commelina benghalensis L.* were collected from Madurai Medical College, Madurai (DT) , during the month of August- 2017 and was authenticated by Dr. D. Stephen, M.Sc., Ph.D., Assistant professor, Department of Botany, American College, Madurai-20. The herbarium of this specimen was kept in the department for further reference.

Preparation of Hydroalcoholic Extract of *Commelina Benghalensis L.*

Procedure

The leaves were collected, shade dried was powdered coarsely and was defatted with petroleum ether (60-80˚c). The residue was dried and extracted with hydroalcohol (70%) by maceration until the complete extraction of the powder was filtered and concentrated under reduced pressure to obtain a solid residue (dark brown). (HAECB)

Invitro Anti-Inflammatory Activity Screening By Membrane Stabilization Study

Hydroalcoholic extract was subjected to n vitro anti inflammatory and anti arthritic activity as per Sadique et al.,Oyedapo et al., [24-27]

PROCEDURE

Preparation of HRBC suspension in isosaline

The human erythrocytes suspension was used for the *in-vitro* membrane stabilization assay. Blood was collected from the healthy volunteers who had not consumed any NSAIDs for two weeks prior to the experiment. The blood was mixed with equal volume of Alsever's solution (2% dextrose, 8.0% sodium citrate, 0.5% citric acid, 0.42% sodium chloride) and centrifuged at 3000 rpm. The packed cells were washed with isosaline and a 10% v/v erythrocyte suspension in isosaline was prepared.

Membrane Stabilization Study

The assay mixture was prepared with 2 ml of hypoosaline and 1 ml of phosphate buffer and varying volumes (0.1 to 0.5 ml) of HAECB extract at different concentration (10, 20, 30, 40, 50 µg/ml) and 0.5 ml HRBC suspension in isosaline, then the final volume were made upto 4.5 ml with isosaline. The control was prepared as mentioned above without the test extract, while product control was also prepared similarly but without HRBC suspension. The reaction mixture was incubated at 56º C for 30 min in a water bath, then the tube was cooled under running water and the absorbance of the released haemoglobin was measured at 560 nm. Diclofenac sodium was used as a reference standard. The percentage membrane stabilization activity of the compounds were determined by the formula

$$\% \text{ Membrane stabilization} = \frac{[\, A_{control} - (\, A_{test} - A_{product\ control}) \,]}{A_{control}} \times 100$$

Where

$A_{control}$ - Absorbance in control

A_{test} - Absorbance in test

$A_{product\ control}$ - Absorbance in product control

The results are displayed in (Figure I) and tabulated in (Table I)

Invitro Antiarthritic Activity By Protein Denaturation Method

Hydroalcoholic extract was subjected to in antiarthritic activity ed by inhibition of protein denaturation method as per Lavanya et al., and Shravan Kumar et al.,[28&29].

Experimental Protocol

The following four solutions were prepared

Test solution (0.5 ml)

The test solution consists of 0.45 ml bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of

HAECB (10, 20, 30, 40, and 50 µg/ml concentration).

Test control solution (0.5 ml)

The test control solution consists of 0.45 ml bovine serum albumin and 0.05 ml distilled water.

Product control (0.5 ml)

The product control consists of 0.45 ml of distilled water and 0.05 ml of HAECB (10, 20, 30, 40, and 50 µg/ml concentration).

Standard solution

Standard solution consists of 0.45 ml of bovine serum albumin and 0.05 ml of Diclofenac sodium solution.

PROCEDURE

All the above test samples was adjusted to p^H 6.3 using a small amount of 1N hydrochloric acid. They were incubated at 37º C for 20 minutes and heated at 57º C for 3 minutes. Allow to cool and about 2.5 ml of phosphate buffer (p^H 6.3) was added to all the above solution. The absorbance was measured using UV spectrophotometer at 416 nm. The percentage inhibition of protein denaturation was calculated using the formula:

$$\text{Percentage inhibition} = 100 - \frac{\text{OD of test solution} - \text{OD of product control}}{\text{OD of test control}} \times 100$$

The control represents 100% protein denaturation. The results were compared with the standard drug diclofenac sodium treated sample.
The results are displayed in (Figure II) and tabulated in (Table II)

RESULTS AND DISCUSSION

Anti-Inflammatory Activity

The inhibitory concentration (IC_{50}) of HAECB in HRBC membrane stabilization study is found to be 69µg/ml in comparison with diclofenac sodium 57µg/ml.

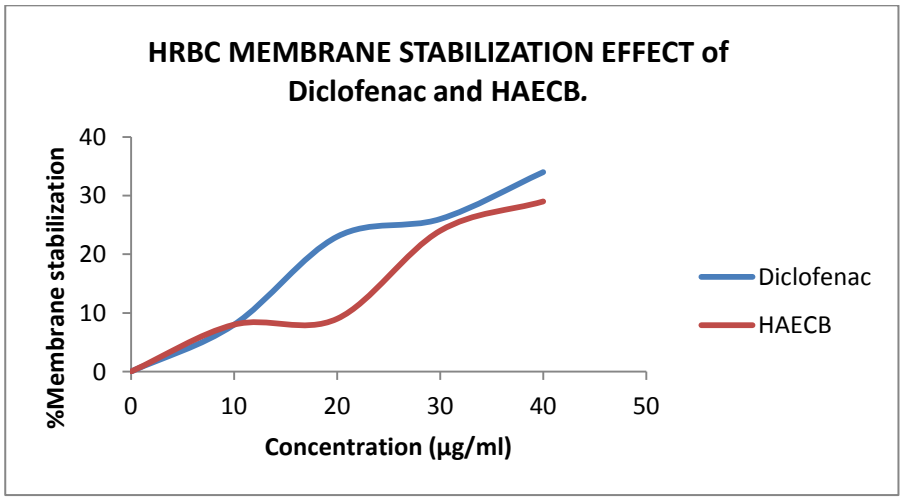

Figure I: Percentage of Membrane Stabilization By Diclofenac Sodium And HAECB

Table I: Percentage of Membrane Stabilization By Diclofenac Sodium and HAECB

S.NO	Concentration(µg/mL)		Percentage Membrane stabilization of Diclofenac	Percentage Membrane stabilization of HAECB
	Diclofenac	HAECB	Mean±SEM*	Mean±SEM*
1	10	10	8.14 ± 0.0088	7.7±0.0577
2	20	20	22.75±0.0088	9 ± 0.0577
3	30	30	25.9 ±0.0088	23.5±0.1453
4	40	40	34.4 ±0.3333	29.3±0.0882
		IC_{50}	**57 µg/mL**	**69 µg/mL**

*Mean of three readings ± SEM

The hydroalcoholic extract of *Commelina benghalensis L.* showed mild anti-inflammatory activity in comparision with diclofenac used as reference..

ANTI-ARTHRITIC ACTIVITY

The inhibitory concentration (IC_{50}) of HAECB in Protein denaturation is found to be 17µg/ml in comparison with diclofenac sodium 14µg/ml.

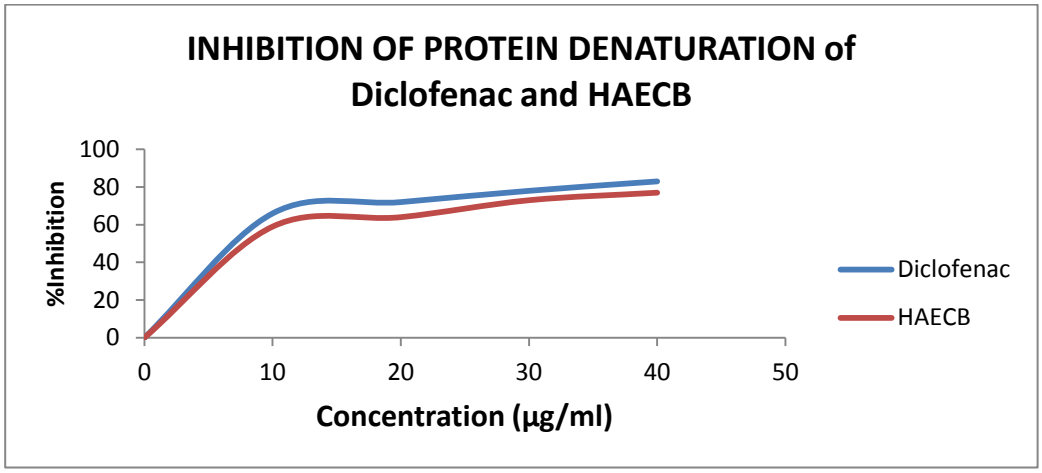

Figure 2 Inhibition of Protein Denaturation of *Commelina benghalensis L.*

Table II: Effect of HAECB And Diclofenac Sodium on Inhibition of Protein Denaturation

S.NO	Concentration (µg/mL)		Percentage Inhibition of Diclofenac	Percentage Inhibition of HAECB
	Diclofenac	HAECB	Mean±SEM*	Mean±SEM*
1	10	10	65.6 ± 0.3464	59.4 ±0.2309
2	20	20	71.9 ± 0.5774	64.1 ±0.0882
3	30	30	78.1 ± 0.0882	73.4 ±0.2309
4	40	40	82.8 ± 0.5774	76.6 ±0.1732
		IC_{50}	**14 µg/mL**	**17 µg/mL**

*Mean of three readings ± SEM

The hydroalcoholic extract of *Commelina benghalensis L.* showed mild anti- arthritic activity in comparision with diclofenac used as reference..

CONCLUSION

At the site of inflammation, HAECB may possibly inhibit the release of lysosomal content of neutrophils (bactericidal enzymes and proteinases) which upon extracellular release cause further tissue inflammation and damage [30]. In the present study, results indicate that the HAECB possesses significant anti-inflammatory properties which may be due to the strong occurrence of polyphenolic compounds such as flavonoids, tannins and phenols. HAECB showed significant antiarthritic activity by inhibition of protein denaturation. HAECB had shown significant anti-arthritic activity and the phenolic constituent may be responsible for this activity.

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