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Research Article

ANTI-INFLAMMATORY ACTIVITY OF FLOWER EXTRACT OF *CASSIA AURICULATA* – AN *IN-VITRO* STUDY

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Abstract: Medicinal plants have provided the modern medicine with numerous plant derived therapeutic agents. *Cassia auriculata* is highly valued in Indian medicines for management of painful inflammation and diabetes. The objective of this study was to evaluate the anti-inflammatory potential of acetone extract of *C. auriculata* flowers. The anti-inflammatory activity was evaluated using albumin denaturation assay, proteinase inhibitory activity and membrane stabilization at different concentrations. Aspirin and Diclofenac sodium were used as standard drugs. The percentage of inhibition was compared with those of standard drugs. The results indicated that the Acteone flower extract *C. auriculata* possess anti-inflammatory properties and so it can be used as a potential source of anti-inflammatory agents.

Key words: *C. auriculata*, Anti-inflammatory activity, Albumin denaturation, Proteinase inhibitory activity, Membrane stabilization, Haemolysis

INTRODUCTION

Cassia auriculata is a evergreen shrub that grows in many parts if India and in other parts of Asia. The flower, leaves, stem, root and unripe fruit are used for treatment, especially in Ayurvedic medicine. People use *C. auriculata* for diabetes, eye infections (conjunctivitis) joint and muscle pain (rheumatism), constipation, jaundice, liver disease and urinary tract disorders. The plant has been reported to possess antipyretic, hepatoprotective, antidiabetic, antiperoxidative and antihyperglyceamic[1] and microbicidal activity. The flowers are used to treat urinary discharges, nocturnal emissions, diabetes and throat irritation[2].

Inflammation is the reaction of living tissues to injury, infection or irritation. Lysosomal enzymes released during inflammation produce a variety of disorders which leads to the tissue injury by damaging the macromolecules and lipid peroxidation of membranes which are assumed to be responsible for certain pathological conditions as heart attacks, septic shocks and rheumatoid arthritis etc. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil such as bacterial enzymes and proteases which cause further tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane[3]. The erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane[4].

The search of new agents having various pharmacological activities, obtained by screening the natural sources like different plant extracts has led to the discovery of many clinically useful drugs which are immensely useful in the treatment of various human diseases[5]. Herbal drugs can therefore be considered as a better alternative to synthetic anti-inflammatory drugs[6]. Previous studies has proved that the chemical constituents such as flavonoids, bioflavonoids, alkaloids, tannins and terpenoids are promising agents in treatment of inflammation[7,8,9]. Flavonoids such as hesperidin, apigenin, luteolin and quercetin are found to be a potent anti-inflammatory constituent[10]. High content of flavonoids and bioflavonoids in methanolic extract of *C.auriculata* flowers is reported for its anti-inflammatory activity.

The present study involves determination of anti-inflammatory activity of *C.auriculata* by Inhibition of albumin denaturation, Antiproteinase action and Membrane stabilization.

MATERIALS AND METHODS

Collection of plant material

The fresh flowers of *C.auriculata* were collected from Panakkudi, Tirunelveli district, Tamil Nadu.

Preparation of extract[10]

The flowers were garbled and dried under shade and powdered. Thirty grams of the flower powder was mixed with 100ml of the acetone in a clean conical flask. The mixture was kept undisturbed for 72hrs in room temperature and stirred every 24hr using a sterile glass rod for perfect mixing. Extract were filtered by using Whatmann No.1 filter paper and solvent was removed by placing in an incubator at 37ºC for 48 hrs until all the solvents get evaporated. The residues under the bottom of the conical flasks were used for anti-inflammatory activity test.

Assessment of invitro anti-inflammatory activity

Inhibition of albumin denaturation

The anti-inflammatory activity of flower extract of *Cassia auriculata* was studied using inhibition of albumin denaturation technique which was studied according to Mizushima *et al*[11] and Sakat *et al*[12] followed with minor modifications. The reaction mixture (2 ml) was containing test extracts of different concentrations (100-500 µg/ml), 100µg/ml Aspirin (Standard anti-inflammatory drug) and 1% aqueous solution of bovine albumin fraction. The sample extracts were incubated at 37ºC for 20min and then heated to 51ºC for 20min, after cooling the samples the turbidity was measured at 660nm. (UV-visible Spectrophotometer). The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (Abs Control – Abs Sample) x 100 / Abs control

Antiproteinase action

The test was performed according to Oyedopo *et al*[13] and Sakat *et al*[12] with minor modifications. The reaction mixture (2ml) was containing 0.06mg trypsin, 20mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations (100-500 µg/ml).The mixture was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210nm against buffer as blank. The experiment was performed as triplicate. The percentage inhibition of proteinase inhibitory activity was calculated.

Percentage inhibition = (Abs Control – Abs Sample) x 100 / Abs control

Membrane stabilization

Preparation of Red Blood cells (RBCs) suspension[12,14]

The blood was collected from healthy human volunteer who has not taken any Non Steroidal Anti-Inflammatory Drugs) for 2 weeks prior to the experiment and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10min and were washed three times with equal volume of normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline.

Heat induced haemolysis[12,15]

The reaction mixture (2ml) consisted of 1 ml test sample of different concentrations (100-500 µg/ml) and 1ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56ºC for 30min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at2500 rpm for 5 min and the absorbance of the supernatants was taken at 560nm. The experiments were performed in triplicates for test samples.

The percentage of inhibition of Haemolysis was calculated as follows:

Percentage inhibition = (Abs Control – Abs Sample) x 100 / Abs control

Hypotonicity- induced haemolysis[16]

Different concentration of extract (100-500µg/ml), reference sample and control were separately mixed with 1ml of phosphate buffer, 2ml of hyposaline and 0.5ml of HRBC suspension. Diclofenac sodium (100µg/ml) was used as a standard drug. All the assay mixtures were incubated at 37ºC for 30 min and centrifuged at 3000rpm. The supernatant liquid was decanted and the haemoglobin content was estimated by a spectrophotometer at 560mm. The percentage haemolysis was estimated by assuming the haemolysis produced in the control as 100%.

Percentage protection = 100-(OD sample / OD control) x 100

RESULTS AND DISCUSSION

Albumin denaturation

Denaturation of proteins is a well documented cause of inflammation. Most biological proteins lose their biological functions when denatured. Production of autoantigen in certain arthritic disease is due to denaturation of protein[17]. The mechanism of denaturation involves alteration in electrostatic hydrogen, hydrophobic and disulphide bonding[18].

It was reported that *Centella asiatica* showing 89.76% inhibitory activity on protein denaturation at 2000 µg/ml[19]. From the result of the present study, it can be stated that the Acetone flower extract of *C. auriculata* (AFCA) is capable of inhibiting denaturation of proteins. Maximum inhibition of 74% was observed at 500 µg/ml. Aspirin, a standard anti-inflammatory drug showed the maximum inhibition of 69% at the concentration of 100 µg/ml.(Table-1)

Proteinase inhibitory action

It was previously reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors[20]. In another study, it was reported that *Enicostemma axillare* methanol extract showed maximum inhibition of 53% at 500µg/ml and Aspirin showed 55% at 100µg/ml[21]. In the present study, AFCA exhibited significant antiproteinase activity at different concentrations as shown in Table 2. It showed maximum inhibition of 66% at 500µg/ml and Aspirin showed maximum inhibition of 55% at100 µg/ml.

Membrane stabilization

During inflammation, lysosomal hydrolytic enzymes are released which causes damages of the surrounding organelles and tissues with attendance variety of disorders. The erythrocyte membrane is analogous to the lysosomal membrane[22] and its stabilization implies that the extract may as well stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases which cause further tissue inflammation and damage upon extracellular release[23].

It is reported that ethanolic extract of *Operculina turpethum* at a concentration of 800µg/ml showed 46.43% protection

of RBC hemolysis in hypotonic solution[24]. In this study, the extract was effective in inhibiting the heat induced and hypotonicity induced hemolysis at different concentrations as shown in Table 3 and 4. In heat induced hemolysis, the results showed that AFCA at 500µg/ml concentration exhibit 64% of inhibition and Aspirin showed 53% inhibition at 100µg/ml concentration.

The hypotonicity induced hemolysis, AFCA showed 65% of inhibition at 500µg/ml and Diclofenac sodium showed 55% inhibition at 100µg/ml concentration.

The mode of action of the extracts and standard anti-inflammatory drugs could be connected with binding to the erythrocyte membranes with subsequent alteration of the surface charges of the cells[25]. It has been reported that certain saponins and flavanoid exerted stabilizing effect on lysosomal membrane both *invivo* and *invitro* while tannins and saponins possess ability to bind cations, thereby stabilizing erythrocyte membranes and other biological macromolecules[26].

Table 1: Effect of AFCA on heat induced protein denaturation

Treatments	Concentration (µg/ml)	Absorbance (660nm)	Inhibition (%)
Control	-	0.39±0.06	-
AFCA	100	0.24±0.03	38
AFCA	200	0.20±0.03	48
AFCA	300	0.16±0.04	58
AFCA	400	0.13±0.01	66
AFCA	500	0.10±0.06	74
Aspirin	100	0.12±0.01	69

Each value represents the mean ± SD

Table 2: Effect of AFCA on proteinase inhibitory action

Treatments	Concentration (µg/ml)	Absorbance (210nm)	Inhibition (%)
Control	-	0.45±0.09	-
AFCA	100	0.32±0.06	28
AFCA	200	0.29±0.02	35
AFCA	300	0.22±0.06	51
AFCA	400	0.19±0.01	57
AFCA	500	0.15±0.03	66
Aspirin	100	0.17±0.01	62

Each value represents the mean ± SD

Table 3: Effect of AFCA on heat induced haemolysis

Treatments	Concentration (µg/ml)	Absorbance (560nm)	Inhibition (%)
Control	-	0.28±0.04	-
AFCA	100	0.20±0.09	28
AFCA	200	0.18±0.08	35
AFCA	300	0.14±0.05	50
AFCA	400	0.12±0.02	57
AFCA	500	0.10±0.03	64
Aspirin	100	0.13±0.06	53

Each value represents the mean ± SD

Table 4: Effect of AFCA on hypotonicity induced haemolysis

Treatments	Concentration (µg/ml)	Absorbance (560nm)	Inhibition (%)
Control	-	0.43±0.02	-
AFCA	100	0.36±0.01	16
AFCA	200	0.32±0.08	25
AFCA	300	0.28±0.05	34
AFCA	400	0.25±0.07	41
AFCA	500	0.15±0.03	65
Diclofenac sodium	100	0.19±0.01	55

Each value represents the mean ± SD

CONCLUSION

In the present study, results indicate that the Acetone flower extract of *C. auriculata* possess anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavanoids, tannins, steroids and phenols. Protective effect

on heat and hypotonic saline, induced erythrocyte lysis is known to be a good index of anti-inflammatory activity of any agent. Since the membrane of RBC is structurally similar to the lysosomal membrane the effect of any substance on stabilization of RBC membrane may be extrapolated to the stabilization of lysosomal membrane. Purification of bioactive compound is necessary which may show increased activity. Hence, this study gives an idea that the compound of plant *C. auriculata* can be used as a lead compound for designing a potent anti-inflammatory drug which can be used to cure inflammation.

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