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Evaluation of Invitro Anti- Inflammatory Activity of Aqueous Leaves Extract of Gliricidia Sepium

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ABSTRACT
Gliricidia sepium (Family – Fabaceae). It is a widespread evergreen, medium sized tree. The present study highlights the invitro anti-inflammatory activityof aqueous leaves extract of Gliricidia sepium. Maceration was used as the extraction method and the phytochemical screening were carried out, the extracts showed the presence of alkaloids, glycosides, tannins, flavonoids, carbohydrates and proteins. In vitro anti-inflammatory studies were done by Protein denaturation method and Proteinase inhibitory method. The aqueous leaves extracts of the plant showed significant Anti- inflammatoryactivitywhen compared with the standard drug.

Keywords: Gliricidia sepium, Protein denaturation method, Proteinase inhibitorymethod.

I. INTRODUCTION

Gliricidia sepium is a medium-sized leguminous tree that grows 10–12 m (33–39 ft.) high. Which belongs to the family Fabaceae.Gliricidia sepium is used in many tropical and subtropical countries for live fencing. The bark is smooth, and its color can range from a whitish gray to deep red-brown. The flowers are pinkish white and can be on the end of branches without leaves. The fruit is a pod which is about 10-15 cm in length. Gliricidia have composite leaves with odd pinnate and usually alternate with an average of 22.68cm long and 3.92cm width. The leavesof Gliricidia sepium grow alternately on the stem (spirally arranged). The leaf is compound(imparipinnate). The leafletsare bright green- colored above and paler beneath. The leaflets are coriaceous and glabrous on both sides (some minute hairs on the midrib beneath). The leaflet has a 3-4mm long petiole. The leaflet of Gliricidia sepium is 7-8 cm long and 2.5-3 cm wide. The shape of the bladeis ovate-elliptic, the apexis apiculate, the baseis oblique and the margins are entire. The venationof the leaf is reticulate with a prominent midrib[1,2,3].

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membranealteration. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form of stress. Inflammation of tissue is due to response to stress. It is defensive response that is characterized by redness, pain, heat, and swelling and loss of function inthe injured area. The most common causes of inflammation are infections, burns and trauma, and many types of immune reactions[4].Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes(especially granulocytes) from the blood into the injured tissues.A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process[5].

II. MATERIALS AND METHODS

Plant Collection and Drying

The leaves of Gliricidia sepium were collected from Kasaragod district, Kerala in the month of April.The plant materials were dried under shade for fewdays, powdered with mechanical grinder and stored in an air tight container.

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Preliminary Phytochemical Screening

The leaves are dried, powdered and subjected to maceration with water for 3 days. This extract of the powdered crude drug was used for anti-inflammatory activity study. These extracts were subjected to phytochemical screening[6].

Anti-Inflammatory Activity[7,8,9]

Inhibition of Protein Denaturation

The reaction mixture (0.5ml) consisted of 0.45ml bovine serum albumin (5% aqueous solution) and 0.05ml leave extract of 50,100,150,200 µg/ml concentrations and pH was adjusted to 6.3 using 1N HCl. The sample were incubated for 37°C for 20 min and then heated at 57°C for 30 min, Ibuprofen used as standard drug (50, 100, 150, 200 µg/ml). After cooling the samples, 2.5mlphosphate buffer saline (pH 6.3) was added to each tube. Absorbance was measured spectrophotometrically at 660nm.

For control tests 0.05ml distilled water was used instead of extracts while product control lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows

PercentageInhibition = (Abs $_{Control}$ − Abs $_{Sample/standard}$)/Abs $_{Control}$× 100

Calculation of IC50 (50% inhibitory concentration)

The concentration (µg/ml) of the drug required to denature 50% protein was calculated from the graph. The IC50 was calculated for concentration of both the sample and standard.

Proteinase inhibitory method

The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1ml 20mM Trypsin HCl buffer (pH 7.4) and 1 ml test sample of different concentration (50, 100, 150 and 200 µg/ml). The mixture wasincubated at 37^0C for 5 min and then 1 ml of 0.8 % (w/v) casein was added. The mixture incubated for 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 210 nm against buffer blank. The experiment was performed in triplicate. Ibuprofen was used as standard (50, 100, 150 and 200 µg/ml). The percentage inhibition of proteinase inhibitory activity was calculated as follows.

Percentage of inhibition= [(A0-A1) / AO] 100

Where,

A0 = Absorbance of the control

A1= Absorbance of the sample / standard

Calculation of IC50 (50% inhibitory concentration)

The concentration (µg/ml) of the drug required to denature 50% proteinase was calculated fromthe graph. The IC50 was calculated for concentration of both the sample and standard.

III. RESULTS AND DISCUSSION

Plant collection and authentication

Gliricidia sepium plant leaves were collected from Kasaragod district and authentified by Dr. Biju P, Assistant Professor Department of Botany, Government College Kasaragod, vidyanagar.Collected plant leaves were dried under shade and stored in air tight container.

Preliminary Phytochemical Screening

The leaves are dried, powdered and subjected to maceration with water for 3 days. This extract of the powdered crude drug was used for anti-inflammatory activity study. These extracts were subjected to phytochemical screening.Preliminary phytochemical screening reveals the presence ofalkaloids, phenolic compounds, flavonoids, glycosides, carbohydrates, tannins and proteins.

Protein denaturation method (Bovine serum albumin)

In this method, the aqueous extract of leaves of plant Gliricidia sepium (50, 100, 150 and 200µg/ml) displayed significant activity. The extracts at a concentration of 200µg/ml showedmaximumactivity.

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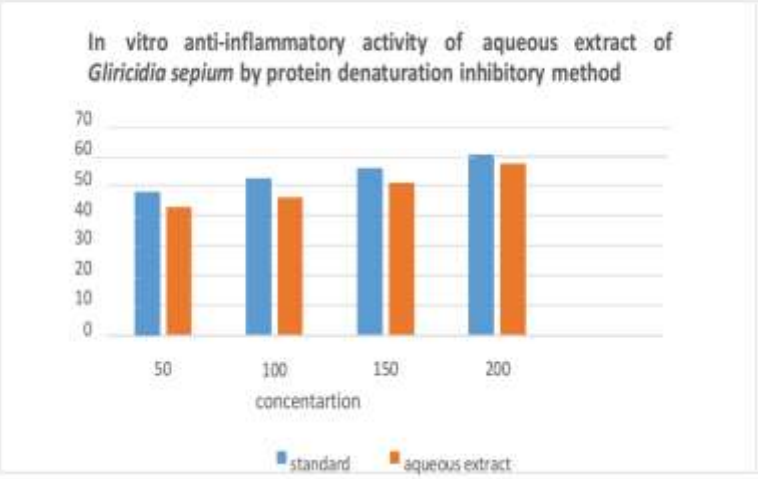
Results of in vitro anti-inflammatory activity of aqueous extract of leaves of Gliricidia sepium by Protein denaturation method

Sl. no	Sample	Concentration (µg/ml)	Absorbance at 660nm	% inhibition
1.	Control	-	0.063	-
2.	Standard	50	0.033	47.61
		100	0.030	52.38
		150	0.028	55.55
		200	0.025	60.31
4..	Aqueous extract	50	0.036	42.85
		100	0.034	46.03
		150	0.031	50.79
		200	0.027	57.14



Anti-inflammatory activity of aqueous extracts of leavesof Gliricidia sepium by Protein denaturation method.

IC50 value was calculated for aqueous extracts of leaves of plant Gliricidia sepium andstandard fromthegraph.

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Results showing IC50 value of aqueous extracts of leaves of Gliricidia sepium by Protein denaturation method

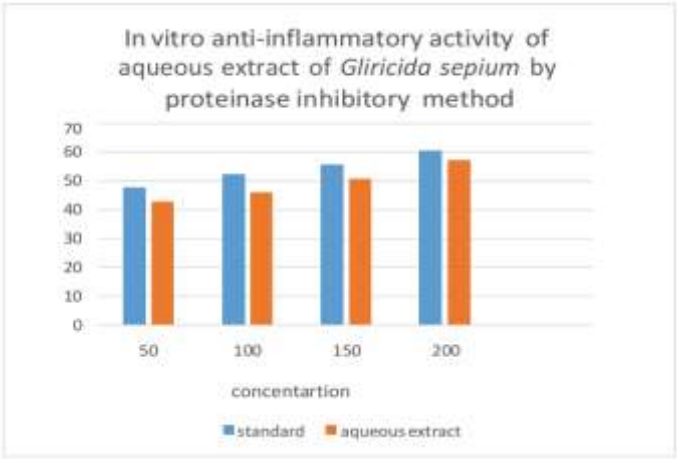
Sl. No	Sample	IC50 (mcg/ml)
1.	Standard	77.56
2.	Aqueous extract	133.78

Proteinase inhibitory method

The proteinase inhibition of aqueos extract of leaves of Gliricidia sepium was performed. Aqueous extracts and standard showed dose dependent activity.

Results showing in vitro anti-inflammatory activityof aqueous extracts of leaves of the plant Gliricidia sepium by Proteinase inhibitory method

Sl no	Sample	Concentration (µg/ml)	Absorbance At230 nm	% Inhibition
1	Control	-	0.568	-
2	Standard	50 100 150 200	0.331 0.246 0.207 0.105	41.72 56.69 63.55 81.51
3	Aqueous extract	50 100 150 200	0.340 0.263 0.218 0.116	40.14 53.69 61.61 79.57



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Results showing in vitro anti-inflammatory activity of aqueous extracts of leaves of the plant by Proteinase inhibitory method

IC50 value was calculated for aqueous extract of leaves of plant Gliricidia sepium and standard from the graph

Results showing IC50 value of various extracts of leaves of the plant Gliricidiasepium byProteinase inhibitory method

Sl. No	Sample	IC50 (µg/ml)
1	Standard	80
2	Aqueous extract	104.07

IV. CONCLUSION

The air dried powder of Gliricidia sepiumsubjectedtomaceration with water for 3 days. The extract obtained from maceration used for preliminary phytochemical analysis and reveals the presence of alkaloids, phenolic compounds, flavonoids, glycosides, carbohydrates etc. The invitro anti-inflammatory activity of the leaves were determined by using proteindenaturation and proteinase inhibitory methods, both the methods shows significant anti-inflammatory activitywhen compared with the standard Ibuprofen drug.

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