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EVALUATION OF *IN VITRO* ANTI INFLAMMATORY ACTIVITY OF ROOT METHANOL EXTRACT OF *PSEUDARTHRIA VISCIDA* (L) WIGHT AND ARN.

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Pseudarthria viscida (L) Wight and Arn. (Leguminosae) commonly called "Moovila", is a perennial viscid pubescent semi erect diffuse under shrub. It is an essential component of many Ayurvedic formulations like Dashamoola, Mahanarayana taila and Dhantara taila. The main aim of the proposed work is to evaluate the anti inflammatory activity of *Pseudarthria viscida* along with preliminary qualitative phytochemical analysis. The anti inflammatory activity was evaluated by cyclooxygenase inhibition assay (COX-2) and 5-lipoxygenase inhibition assay (5-LOX) in Human Platelet lysate (HPL'S) culture. The results revealed that percentage of inhibition was increasing with the increase in the concentration of the sample. The percentage of inhibition was higher in COX-2 assay than 5-LOX assay. The present study thus confirmed that *Pseudarthria viscida* could be used as potent anti inflammatory agent. Bioactive molecules present in roots could be the reason for high anti inflammatory activity. So further studies are necessary to evaluate and isolate the active principles responsible for anti inflammatory activity.

Keywords : Anti inflammatory; Cyclooxygenase assay; Leguminosae; 5- Lipoxygenase assay; *Pseudarthria viscida.*

Introduction

One of the most important complex responses of our body is inflammation. The classic manifestations of the inflammatory process include pain, heat, redness and swelling. Prostaglandins act as short lived localized hormones that can be released by any cell of the body during tissue, chemical, or traumatic injury and can induce fever, inflammation and pain; once they are present in the intercellular space[1]. The inflammatory process consists of sets of signal cascades, each signal or molecule is responsible for mediating the upper or lower level of inflammatory response. By blocking or trapping each signal molecule, or triggering stop signal, the inflammatory process can be inhibited or suppressed[2].

Cyclooxygenase (COX) is the key enzyme that catalyzes the rate limiting step in prostaglandin synthesis, converting arachidonic acid into prostaglandin H2, which is then further metabolized to prostaglandin E2 (PGE2), prostaglandin F2 (PGF2), prostaglandin D2 (PGD2) and other eicosanoids (3). COX-1 is constitutively expressed in many tissues and plays a role in tissue homeostasis. COX-2 which can be expressed in a variety of cells and tissues is an inducible isoform which is stimulated by growth factors, inflammatory cytokines, carcinogens, and tumor promoters, implying a role for COX-2 in both inflammation and control of cell growth. Thus, compounds that inhibit the activity or expression of COX-2 might be an important target for antiinflammation[3]. Another enzyme lipoxygenases are a family of iron containing enzymes that catalyse the dioxygenation of poly unsaturated fatty acids in lipids containing a *cis,cis*-1,4-pentadiene structure. Lipoxygenases convert arachidonic acid into leukotrienes, which is then further metabolized to leucotriene A4, leucotriene B4 and cysteinyl leucotrienes. These are called proinflammatory mediators[4].

Chemical reagents with anti inflammatory activity may reduce the incidence of various diseases derived from inflammation. Although anti inflammatory drugs are used extensively, prolonged consumption of these medications is usually coupled with numerous side effects. Therefore, there is a need to explore alternative strategies to lower the formation of inflammatory mediators with the help of natural dietary products[5]. The potential importance of medicinal plants has been internationally recognized[6].

Pseudarthria viscida (L) Wight and Arn. is a perennial viscid pubescent semi erect diffuse under shrub, belonging to the family Leguminosae. It is distributed

Table 1. Effect of methanol root extract of *Pseudarthria viscida* on Cyclooxygenase inhibition assay.

Sample Concentration	:	Absorbance (at 632nm)	Percentage of Inhibition
Control		0.118	No inhibition
Standard Aspirin	: (100 μgmL^{-1})	0.012	95.34
Pseudarthria viscida-			
root methanol extract	: 100 μgmL^{-1}	0.081	31.3
	: 500 μgmL^{-1}	0.027	77.11
	: 1000 μgmL^{-1}	0.015	87.23

Table 2 . Effect of methanol root extract of *Pseudarthria viscida* on Lipoxygenase inhibition assay.

Sample Concentration	:	Absorbance (at 632nm)	Percentage of Inhibition
Control	:	0.130	No inhibition
Standard Aspirin	: (100 μgmL^{-1})	0.016	96.38
Pseudarthria viscida-			
root methanol extract	: 100 μgmL^{-1}	0.122	6.15
	: 500 μgmL^{-1}	0.077	40.76
	: 1000 μgmL^{-1}	0.055	57.69

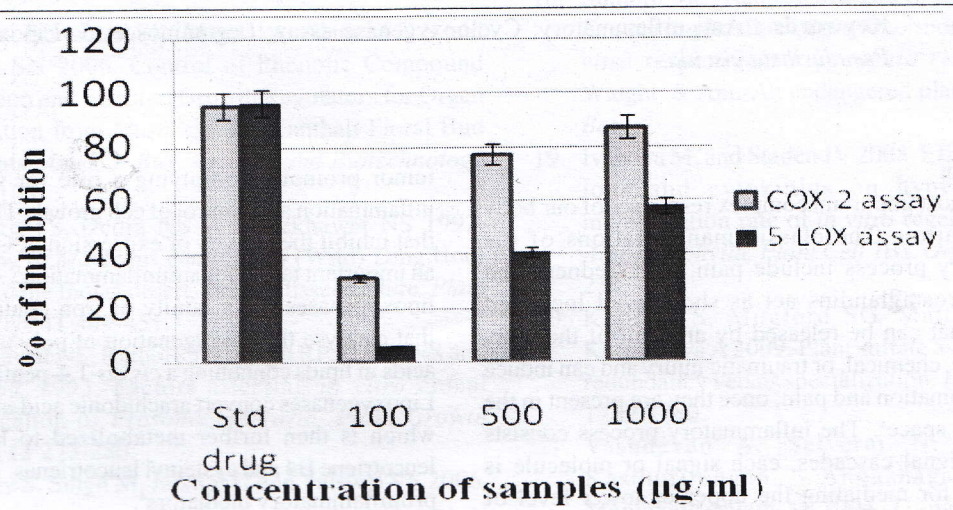

Fig.1. Effect of methanol root extract of *Pseudarthria viscida* on COX-2 and 5-LOX inhibition assay.

throughout India especially found in river basins and in hills up to above 900m[6]. The roots are astringent, thermogenic, digestive, anthelmintic, anti inflammatory, anti fungal, anti diarrhoel, anti oxidant, aphrodisiac, nervine, febrifuge, cardio and rejuvenating tonic[7,8]. They are useful in vitiated conditions of cough, bronchitis, asthma, tuberculosis, helminthiasis, cardiopathy, fever, hemorrhoids, gout, diabetes, hyperthermia and general debility[9-11]. Major chemical compounds reported to be present in the roots are1.5 dicaffeoyl quinic acid, oleic acid, tetradecanoic acid, rutin, quercetin, gallic acid, ferulic acid and caffeic acid[12]. The present investigation was undertaken to evaluate the *in vitro* anti inflammatory activity of root methanol extract of *Pseudarthria viscida.*

Material and Methods

Preparation of root methanol extract: Root of *Pseudarthria viscida.* (L) Wight and Arn was collected from the Botanical garden of University College. Thiruvananthapuram and shade dried. The dried powdered root (50g) was defatted with petroleum ether (60 to 80°C) by hot extraction method in a soxhlet apparatus to remove waxy substances. The defatted powder material was further

extracted with methanol for 72 h. Concentrated methanol extract was used for preliminary phytochemical studies and anti inflammatory analysis.

Reagents and Chemicals : Tris HCl (pH 8), Heamoglobin, Arachidonic acid, TCA in HCl, Thiobarbituric acid (TBA), Sodium phosphate buffer, Sodium linolente, EDTA, Plant Extracts, LPS (Lipopolysaccharide), Penicillin, FBS (Fetal Bovine Serum) and Aspirin.

Preliminary phytochemical studies: The preliminary phytochemical study of crude methanolic root extract of *Pseudarthria viscida* was done for the detection of phytoconstituents, using standard chemical tests[13-15].

In vitro Anti Inflammatory Activity

Lymphocyte culture preparation: Human Platelet lysate (HPL's) was cultured in RPMI 1640 [HIMEDIA] media, supplemented with 20% heat inactivated Fetal Bovine Serum (FBS), and 20% antibiotics (Penicillin). The culture was filtered using 0.2 µm pore sized cellulose acetate filter (Sartorios) in completely aseptic conditions. Fresh plasma was aseptically added to the culture at a concentration of $1x10^6$ cellsmL^{-1}. The culture was then incubated for 72 hrs. Then the culture was activated by adding 1µL LPS (LPS is a principle component which stimulates the production of inflammation in cultured cell). Standard drug (Aspirin) was added in the concentration of 100µgmL^{-1} from a stock of 100mgmL^{-1} and the sample was added in the concentration of 100µgmL^{-1}, 500µgmL^{-1} and 1000 µgmL^{-1} from a stock of 100 mgmL^{-1} and then incubated for 24 hours [16,17].

Assay of Cyclooxygenase inhibition (COX-2 assay): The isolation of human platelet lysate was done by spinning at 6000 rpm for 10 min. Supernatant was discarded and 200µL of cell lysis buffer (1MTris HCl, 0.25M EDTA, 2M NaCl, 0.5% Triton) was added. The incubation was done for 30 minutes at 4°C and the assays were done in pellet suspended in a small amount of supernatant. The assay mixture contained Tris- HCl buffer, glutathione, hemoglobin and various concentration of root extract. This assay mixture was then added in to the pellet. The reaction was started by the addition of arachidonic acid and terminated after 20 min incubation at 37°C by addition of 0.2mL of 10% TCA in 1N HCl, followed by mixing and addition of 0.2mL TBA. Contents were heated in a boiling water bath for 20 min, cooled and centrifuged at 1000 rpm for 3 min. The supernatant was measured at 632nm for COX activity[16, 17].

% of inhibition was calculated using the formula: (C-T/ C) ×100

(C =Optical density of control, T = Optical density of Test)

Assay of 5-Lipoxygenase inhibition (5-LOX assay): Seventy mg of linoleic acid and equal weight of tween 20 was dissolved in 4mL of oxygen free water and mixed back and forth with a pipette avoiding air bubbles. Sufficient amount of 0.5N NaOH was added to yield a clear solution and then made up to 25mL using oxygen free water. This was divided into 0.5mL portions and flushed with nitrogen gas before closing and kept frozen until needed. The isolation of human platelet lysate was done by spinning at 6000 rpm for 10 min. Supernatant was discarded and 200µL of cell lysis buffer (1MTris HCl, 0.25M EDTA, 2M NaCl, 0.5% Triton) was added. The incubation was done for 30 minutes at 4°C and the assays were done in pellet suspended in a small amount of supernatant. The reaction was carried out in a quartz cuvette at 25°C with 1cm light path. The assay mixture contains 2.75mL tris buffer of pH 7.4, 0.2mL of sodium linoleate and various concentrations of the extracts. The increase in OD was measured in 234nm[18,19] and percentage of inhibition was calculated.

Results and Discussion

Preliminary phytochemical studies: Methanol extract of root of *Pseudarthria viscida* was screened for the presence of various constituents employing standard screening tests. Conventional protocols detected the presence of important secondary metabolites such as glycosides, flavanoids, alkaloids, terpenoids and tannins which could be the reason for high anti inflammatory activity of the plant.

In vitro Anti Inflammatory Activity - The results revealed that the methanol extracts of *Pseudarthria viscida* (L) Wight and Arn. possessed good anti inflammatory activity. The extract was taken in various concentrations and its percentage inhibition was compared with standard drug Aspirin. Percentage of inhibition was increasing with the increase in the concentration of the sample. In the cyclooxygenase inhibition assay (COX-2 assay), the percentage of inhibition were found to be 31.3%, 77.11% and 87.23% for sample concentration 100µgmL^{-1}, 500µgmL^{-1}, 1000µgmL^{-1}, respectively and in the lipoxygenase inhibition assay (5-LOX) percentage inhibition were found to be 6.15%, 40.76% and 57.69%. (Table 1&2 : Fig.1).

Medicinal plants are rich source of secondary metabolites that are claimed to reduce inflammation. The vast number of secondary metabolites which are present in methanol root extract of *Pseudarthria viscida* such as glycosides, flavanoids, alkaloids, terpenoids and tannins. Of these metabolites flavonoids, terpenoids and tannins are known to promote anti inflammatory activity in some plants such as *Solanum nigrum, Citrullus colocynthis,* four

Bolivian *Baccharis* species and *Syzgium cumini*[20-23].

The antinflammatory properties of *Pseudarthria viscida* has been attributed to suppression of prostaglandins (PGs) and leucotrienes synthesis. One of the most important enzyme responsible for the conversion of arachidonic acid to prostaglandins is cyclooxygenase (COX). Overexpression of COX-2 leads to many problems especially inflammation, carcinogenesis of tumors of the colon and rectum[24]. Inhibitors of COX-2 activity are useful for treating inflammation, treating cancer etc. LOX metabolites have also been shown to promote inflammation, tumor cell adhesion stimulate the spreading of tumor cells and augment metastatic potential[25,26].

In the present study, methanol root extract of *Pseudarthria viscida* was capable of exerting inhibitory action on enzymes of the arachidonate cascade in human's cellular systems. Methanol extracts of *Pseudarthria viscida* exert a significant effect on the COX 2 inhibition assay. The methanolic root extract showed high percentage of inhibition in COX 2 inhibition assay was already reported in other plant such as *Viburnum punctatum* by Ilango and Renjith[1]. Methanolic root extract of *Pseudarthria viscida* was reported to have anti inflammatory activity against carrageenan induced rat paw oedema[5]. The present study confirmed anti inflammatory potential of *Pseudarthria viscida* by COX-2 and 5-LOX inhibition assay.

The results of the present study revealed that methanolic root extract of *Pseudarthia viscida* exhibit good anti inflammatory activity in both cyclooxygenase inhibition assay as well as lipoxygenase inhibition assay. Secondary metabolites present in roots could be the reason for high anti inflammatory activity. So the methanolic root extract of *Pseudarthia viscida* should be subjected to further isolation and purification to identify the potent phytochemical constituent responsible for exhibiting marked antiinflammatory activity.

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