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Anti-inflammatory activity of methanolic extract of *Stylosanthes fruticosa* (retz.) leaves

[1] **Budda Nageswaraiah*** [2] **Neerugatti dorababu,** [2]**Ganga Rao Battu,** [1] **Sreerama Satyanarayana,** [1] **Nagendra Y and Mohan B.**

[1] Sri Lakshmi Venkateswara Institute of Pharmaceutical Sciences, Kottapeta, Proddatur, Kadapa, Andhra Pradesh, India.

[2] Andhra University College of Pharmaceutical Sciences, Visakhapatnam, Andhra Pradesh, India.

***Corresponding Author:** E-Mail: nagpharmacy25@gmail.com

ABSTRACT

To evaluate the anti-inflammatory activity of the leaves of *Stylosanthes fruticosa.* Anti-inflammatory activity was conducted by using Carrageenan induced rat paw oedema model. The thickness of paws of rats was measured every hour up to 5 hrs for methanolic extract at doses of 100, 200 and 400mg/kg by volume displacement method, using plethysmometer. All doses of the extract (methanol) from the leaves of showed significant percentage of inhibition on Carrageenan induced paw oedema; methanol extract from the leaves of at dose 400mg/kg produced significant ($P<0.001$) percentage of inhibition when compared to other doses. *Leaves* extract (methanol), showed significant anti-inflammatory activity.

Kevwords: *Stvlosanthes fruticosa. Leaves. Anti-inflammatorv activitv.*

1. INTRODUCTION

Inflammation is the response to injury of cells and body tissues through different factors such as infections, chemicals, and thermal and mechanical injuries[1]. At the very early stage of inflammation, neutrophils are the first cells that migrate to the inflammatory sites under the regulation of molecules produced by rapidly responding macrophages and mast cells predisposed in tissues[2]. As the inflammation progresses, various types of leukocytes, lymphocytes, and other inflammatory cells are activated and attracted to the inflamed site by a signaling network involving a great number of growth factors, cytokines and chemokines[2]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most clinically important medicine used for the treatment of inflammation-related diseases like arthritis, asthma, and cardiovascular disease[3]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications due to their efficacy for a wide range of pain and inflammatory conditions[4]. However, the long-term administration of NSAID may induce gastro-intestinal ulcers, bleeding, and renal disorders due to their nonselective inhibition of both constitutive (COX-1) and inducible (COX-2) isoforms of the cyclooxygenase enzymes[5]. Therefore, new anti-inflammatory and analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates[6]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs[7]. In the present study different doses of methanolic extract of *Stylosanthes fruticosa*, leaves were investigated for anti-inflammatory activity.

2. MATERIALS AND METHODS

2.1. Preparation of extracts from the leaves of *Stylosanthes fruticosa*

The leaves of *Stylosanthes fruticosa* were collected from Tirumala hills, Chitoor, Andhra Pradesh, India and authenticated by Dr. Madhava shetty, taxonomist, Department of Botany, Sri Venkateswara University, Andhra Pradesh. Shade dried leaves of were powdered and separately extracted in a Soxhlet apparatus for 6 hrs with methanol and then concentrated under vacuum at temperature of 45oc by using rotary evaporator, dried completely, weighed and stored in desiccators.

2.2. Drug and Chemicals

Ibuprofen, Carboxy methyl cellulose sodium (NaCMC) and Carrageenan were purchased from Sigma chemicals, USA. All chemicals used in the study were of analytical grade.

2.3. Animals

Albino rats (National Institute of Nutrition, Hyderabad, India) of either sex weighing 200-250gm were used in the studies. The animals were maintained under standard laboratory conditions at an ambient temperature of 23±2°C having 50±5% relative humidity with 12h light and dark cycle. The use and care of the animals in the experimental protocol has been approved by the local Institutional Animal Ethics Committee (Regd. No. 516/01/A/CPCSEA) following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Table1. Anti-inflammatory activity of *Stylosanthes fruticosa* extracts on Carrageenan induced paw edema in rats

Treatment	Dose	Volume of mercury displaced in ml at various time intervals in hours.					
		0	1	2	3	4	5
Control	0.1 ml 1 % w/v	0.39± 0.025	0.49± 0.057	0.62± 0.057	0.65± 0.057	0.67± 0.057	0.69± 0.058
Ibuprofen	10 mg/ kg	0.25± 0.005	0.29±*** 0.005	0.33±*** 0.002	0.25±*** 0.003	0.25±*** 0.003	0.19±*** 0.04
Stylosanthefruticosa Methanolic extract	100 mg/kg	0.36± 0.01	0.45±* 0.02	0.55±** 0.05	0.43±*** 0.03	0.43±*** 0.01	0.41±*** 0.04
Stylosanthe fruticosa Methanolic extract	200 mg/ kg	0.34± 0.01	0.43±** 0.02	0.49±** 0.05	0.37±** 0.03	0.37±*** 0.01	0.35±*** 0.04
Stylosanthe fruticosa Methanolic extract	400 mg/ kg	0.31± 0.01	0.39±*** 0.02	0.45±*** 0.05	0.34±*** 0.03	0.34±*** 0.01	0.33±*** 0.04

Significance of difference in control and extracts treated groups were determined by one –way analysis of variance (ANOVA) *** P<0.001 **P<0.01, * P<0.05 are significant and ^{NS} P> 0.05, are not significant. All values are means of individual data obtained from six rats (n=6).

Table 2. Percentage inhibition of *Stylosanthes fruticosa* bark extracts on Carrageenan induced paw edema in rats

Treatment	Dose	% Inhibition of paw volume at various time intervals					
		0	1	2	3	4	5
Control	0.1 ml 1 % w/v	00	00	00	00	00	00
Ibuprofen	10 mg/ kg	00	40	46	61	62	72
Stylosanthes fruticosa Methanolic extract	100 mg/ kg	00	8	11	33	35	40
Stylosanthes fruticosa Methanolic extract	200 mg/ kg	00	12	20	43	44	49
Stylosanthes fruticosa Methanolic extract	400 mg/ kg	00	20	27	47	49	52

2.4. Acute inflammation model: Carrageenan induced rat paw oedema

Inflammation was induced in the left hind paw of each rat by sub- plantar injection of 1% carrageenan suspension (0.1ml). The right hind paw of the rat was injected 0.1ml of saline. Group-I received drug vehicle (1% sodium CMC). Group-II received standard drugIbuprofen at a dose of 10 mg/kg b.w. Group-III, IV and V received orally methanolic extracts of leaves at doses of 100mg/kg 200mg/kg and 400mg/kg b.w in sodium carboxy methyl cellulose suspension one hour prior to the induction of oedema by carrageenan injection. The paw volume of rats was measured at 0 hr and hourly intervals up to 5th hour after the administration of carrageenan by volume displacement method, using plethysmometer[8].

The percent inhibition of paw thickness was calculated using the formula:

Percentage inhibition = 100[1- (Yt / Yc)]

Yt = Average increase in thickness of paw in groups tested with test compounds.

Yc= Average increase in thickness of paw in control

2.5. Statistical Analysis

Data of Paw thickness was analyzed by using One-Way ANOVA followed by post hoc test Dunnett's test using Graph pad Prism-5 software. The results were expressed as Mean ±S.E.M. $P<0.05$ was considered as significant.

3. RESULTS

3.1. Carrageenan induced rat paw oedema

leaves extracts at 100 mg/kg, 200 mg/kg and 400mg/kg doses and Ibuprofen (10 mg/kg b.w.) inhibited the maximal paw oedema response by 17, 24, 57 and 72 respectively during the 5 h of the carrageenan-induced rat paw acute inflammation. Volume of mercury displaced in ml at various time intervals in hours is recorded. The results of were shown in Table 1and 2. Figure1.

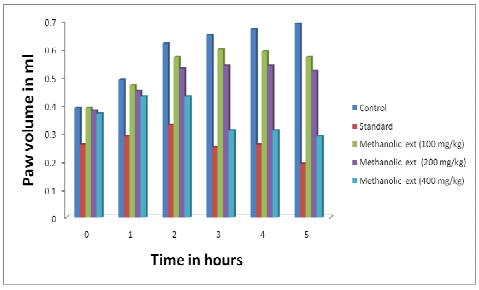

Figure - 1: Histogram showing the anti-inflammatory activity of various extracts of *Stylosanthes fruticosa*

3. DISCUSSION

Conventional NSAIDs of modern medicine are confronted with common and serious unacceptable side effects as well as adverse effects notably gastrointestinal bleeding, hepato nephrotoxicity[3]. Carrageenan induced edema is commonly used as an experimental animal model for acute inflammation and is believed to be biphasic, of which the first phase is mediated by the release of histamine and 5-HT followed by kinin release and then prostaglandin in the later phase[9]. Prostaglandin-E2 (PGE2), a powerful vasodilator, synergizes with other inflammatory vasodilators such as histamine, bradykinin and serotonin and contributes to redness as well as increased blood flow in areas of acute inflammation.

The results in the study indicate that pretreatment with the selected plant extracts and ibuprofen suppressed the increased paw oedema produced by the phlogistic agent. Phytochemical screening confirmed the presence of flavonoids, tannins and saponins in the methanolic extract of leavess of *Stylosanthes fruticosa*

Flavonoids have been reported to play role in analgesic activity by targeting prostaglandins and are known to inhibit prostaglandin synthetase, more specifically the endoperoxides [10]. The results obtained, scientifically support the folklore pertaining to the medicinal properties of *Stylosanthes fruticosa.*

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