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Anti- inflammatory and analgesic activity of *Smilax chinensis*

S.Raghunadha Reddy[1]*, Pobba Rajani[1], Koteswararao. Divi[1, 2], YK Naidu[2,] Sarath Chandiran I[3] and MP Kalyan Reddy[2, 4].

[1] Department of Pharmacy, Annamalai University Chidambaram, Tamil Nadu, India.
[2] Clinical Research and Biosciences (I) Pvt. Ltd., Hyderabad, Andhra Pradesh, India
[3] Gokula Krishna College of Pharmacy, Sullurpet, Andhra Pradesh, India
[4] Jawaharlal Nehru Technological University, Anantapur, Andhra Pradesh, India

ABSTRACT

To evaluate the anti-inflammatory and analgesic activities of ethyl acetate and methanolic extracts of *Smilax chinensis* Linn in rodent models. The anti inflammatory and analgesic activities of ethyl acetate and methanolic extracts were studied by Carragennan induced paw edema method and eddy's hot plate method. *Smilax chinensis* nsis was evaluated for anti-inflammatory action by carrageenan-induced rat paw edema. The analgesic activity was tested by eddy's hot plate method. The ethyl acetate extract of *Smilax chinensis* in doses of 250mg/kg and 400mg/kg showed 68.23% and 73.53% inhibition of paw edema respectively. The methanolic extract of *Smilax chinensis* in doses of 250mg/kg and 400mg/kg showed 56.0% and 61.56% inhibition of paw edema respectively. The ethyl acetate and methanolic extracts from dried rhizome of *Smilax chinensis* Linn (Smilacaceae) revealed significant anti inflammatory and analgesic activities. These results suggest that the ethyl acetate and methanolic extracts of *Smilax chinensis* Linn possesses analgesic and anti inflammatory activities.

Key words: *Smilax chinensis*, Rhizome, Analgesic; Anti-inflammatory

*Corresponding author
Email ID: raghu_nathseelam@yahoo.co.in
Telephone number: +91-9912463912

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INTRODUCTION

Smilax chinensis Linn (Chobchini) [1] is an indigenous herb belonging to the family Smilaceae. *Smilax chinensis* is a deciduous climber with rounded leaves and red berries. *Smilax chinensis* has a hard, large, knotty, uneven rhizome blakish externally, Pale colored or whitish internally shown in Fig.1. The root tubes of which furnish the drug known as china root. It is found in the south Indian states namely Andhra Pradesh, Karnataka and Tamil Nadu. This plant is known to possess analgesic, antiallergic, antiasthmatic, antibiotic, antifungal, anti-inflammatory, antirheumatic, carminative, depurative, diaphoretic, diuretic, febrifuge, hepatoprotective and immunomodulatory activities. The study was undertaken to evaluate the a) antiinflammatory potential [2] of the ethyl acetate and methanolic extracts of Smilax chinensis on carrageenan-induced rat paw edema [3] and b) analgesic activity using Eddy's [4] hot plate method in albino rats.

MATERIALS AND METHODS

Plant material

The dried rhizome part of *Smilax chinensis* were purchased from the local areas of Chidambaram and authenticated by comparing with the voucher specimen present in the department herbarium and with the help of qualified botanist at Annamalai University, Chidambaram.

Extraction

The Rhizomes of *Smilax chinensis* Linn were shade dried, crushed in a mechanical grinder to fine powder of mesh 40. The powder (550 g) was then extracted successively with ethyl acetate and methanol using a soxhlet apparatus. Resulting extracts were filtered, concentrated on water bath, and dried in vacuum drier. The percentage yield with ethyl acetate and methanol was 5 % and 7 % respectively.

Phytochemical screening

The preliminary phytochemical investigation was carried out for the two different extracts obtained from the crude drug. It revealed the presence of Alkaloids, Flavanoids, Gums & Mucilage, Glycosides, Saponins, Terpenes and Tannins.

Animals

Healthy adult male Wister rats weighing (150 - 180) grams were selected for the studies. Animal were obtained from the animal house [5] of Rajah Muthiah Medical College and Hospital, Annamalai University. Animals were allowed to be acclimatized for a period of 10 weeks in our laboratory environment prior to the study. Rats were housed in polypropylene cages (3 animals per cage), maintained under standard laboratory conditions (i.e. 12:12 h light and dark sequence; at an ambient temperature of 25 ± 1ºC). The animals were fed with standard pellet diet and water ad libitum. Institutional Animal Ethics Committee approved the study Protocol (Animal house Reg. No. 160/1999CPCSEA).

Instrument

Plethysmometer, it is glass tube of 20 mm internal diameter and one end fabricated to a glass tube with 0.5 mm bore. This tube is fused to a flexible tube and a pump (glass - syringe) and fixed to other end of the tube. This pump is used to adjust the level of mercury in both the flexible tube and graduated glass tube up to zero level.

Chemicals

Methanol, Ethyl acetate and Tween 80 were purchased from Sigma Chemicals Ltd, Mumbai. Pentazocine from Ranbaxy, Delhi. Indomethacin from Ranbaxy Laboratory Ltd., Chemicals Division, Delhi and Paracetamol from Burroughs welcome, Mumbai.

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Procedure

Anti – inflammatory activity

Assay [6] was performed as described by Winter et al., 1962. Six groups of each six animals were used. Paw swelling was elicited with 0.1 ml carrageenan in 1% saline (w/v) injected in the right hind foot under the plantar aponeurosis. Group-I received 4 % tween80 10ml/kg i.p., Served as Control. Group-II received Indomethacin 20 mg/kg, Served as Standard. Group-III & IV received Ethyl acetate and Methanolic extract of Smilax Chinensis (250 mg/kg i.p), and Group V & VI received Ethyl acetate and Methanolic extract of Smilax Chinensis (400 mg/kg i.p), Served as Tests respectively. All the animals in III, IV, V and VI groups were received their respective doses of the test drug 30 minutes prior to the administration of carrageenan 0.1 ml of 1 % w/v solution.

The inflammation was quantified by measuring the volume displaced by the paw, using a plethysmometer (Ugo Basile) at time 0, 1, 2, 3, 4 & 5 hours after carrageenan injection. The difference between the left and the right paw volumes (indicating the degree of inflammation) was determined and the percent inhibition of edema was calculated in comparison to the control animals. Results are expressed as mean ± S.E.M. Difference between the control and treated group were tested for significance using a one- way analysis of variance (ANOVA) followed by Dunnett's or T-tests. P value less than 0.05 were considered as indicative of significance.

Analgesic activity

The hot plate was used to measure response latencies according to the method described by Eddy's and Leimbach, with minor modifications. The mice were placed on an Ugo basile hot- plate maintained at 56°c and the shaking or licking of the paws or jumping was recorded as the hot-plate latency. Rats with baseline latencies higher than 10 Sec were eliminated from the study. Twenty–four hours later animals were treated with the ethyl acetate and methanolic of 250 & 400 mg/kg i.p Smilax Chinensis respectively before the test. Control animals received the same volume of saline solution (10 ml/kg).

RESULTS AND DISCUSSION

The ethyl acetate and methanolic extract of smilax chinensis were tested in vivo for their ability to reduce the anti-inflammatory response in carrageenan induced paw edema in rats. The effect of *Smilax Chinensis* extract (250 mg/kg i.p) on carrageenan induced paw edema is shown Table.1 and graphically represented in Fig.2 and the effect of *Smilax Chinensis* extract (400 mg/kg i.p) on Carrageenan induced Paw edema is shown Table.2 and graphically represented in Fig.3. The effect of ethyl acetate and methanolic extract of smilax chinensis were tested on mices by Hot Plate Test. The effect of *Smilax Chinensis* extract (250 mg/kg i.p) on Hot plate reaction time is shown Table.3 and graphically represented Fig.4. The effect of ethyl acetate and methanolic extract of smilax chinensis were tested on mices by Hot Plate Test. The effect of *Smilax Chinensis* extract (400 mg/kg i.p) on Hot plate reaction time is shown Table.4 and graphically represented Fig.5.

Fig.1. Rhizome of *Smilax chinensis*

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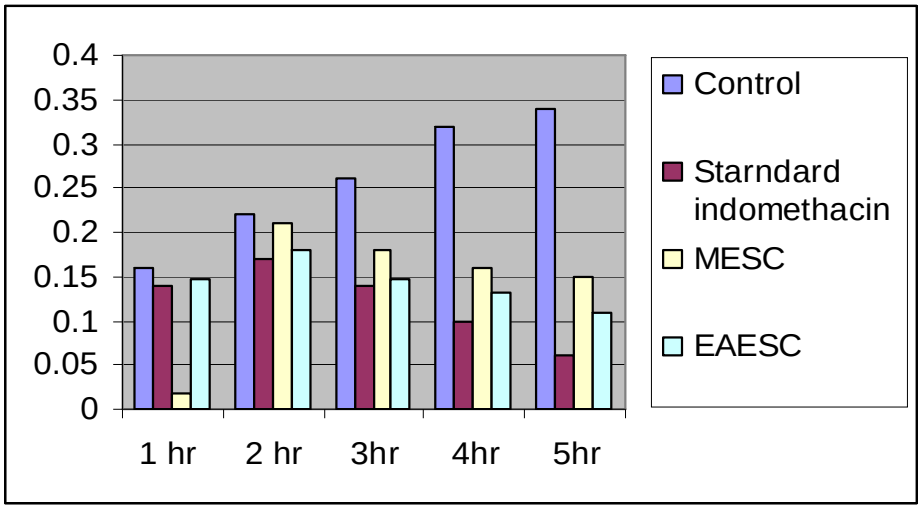

Fig.2.Graphical representation of effect of Smilax Chinensis extract (250 mg/kg i.p) on Carrageenan induced Paw edema.

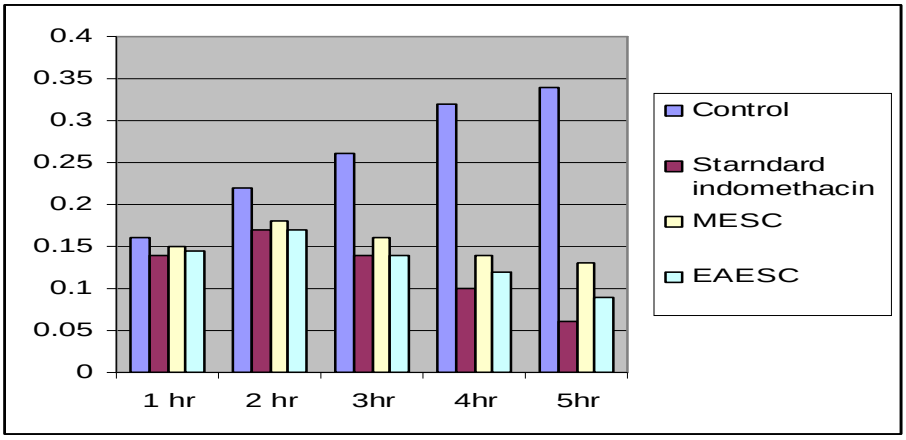

Fig.3.Graphical representation of effect of *Smilax Chinensis* extract (400 mg/kg i.p) on Carrageenan induced Paw edema.

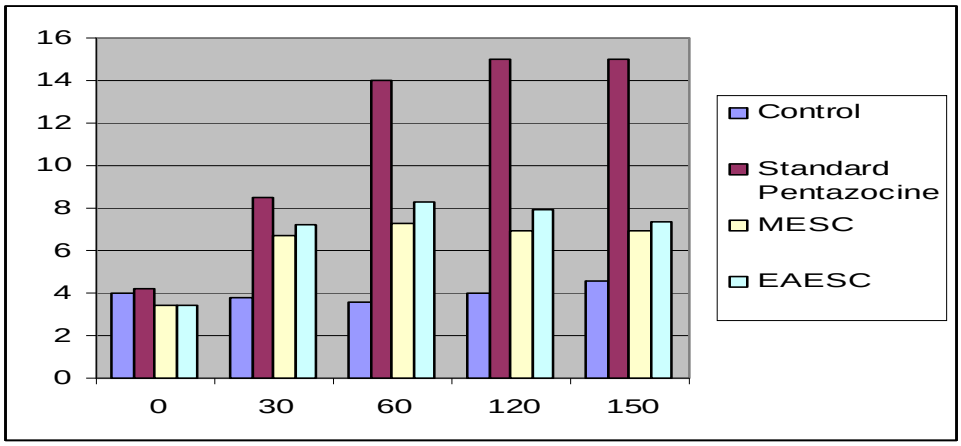

Fig.4. Graphical representation of effect of *Smilax Chinensis* extract (250 mg/kg i.p) on Hot plate reaction time.

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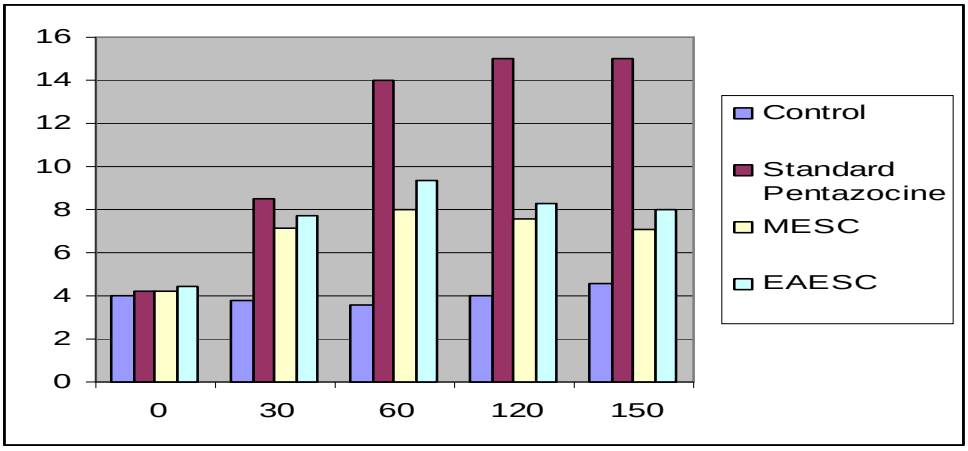

Fig.5.Graphical representation of effect of Smilax Chinensis extract (400 mg/kg i.p) on Hot plate reaction time.

Table.1. Effect of *Smilax Chinensis* extract (250 mg/kg i.p) on Carrageenan induced Paw edema.

Group	Treatment	Edema Volume (ml)				
		1 hr	2 hr	3hr	4hr	5hr
I	Control 4% Tween 80, (10ml/kg)(i.p)	0.16 ± 0.007	0.22 ± 0.007	0.26 ± 0.007	0.32 ± 0.007	0.34 ± 0.007
II	Standard Indomethacin 20mg/kg (i.p)	0.14 ± 0.007$^{\#}$ (12.5%)	0.17 ± 0.012$^{\#}$ (22.73%)	0.14 ± 0.012* (46.15%)	0.10 ± 0.012* (468.75%)	0.06 ± 0.012* (82.35%)
III	MESC (250 mg/kg) (i.p)	0.017 ± 0.004^{ns} (5%)	0.21 ± 0.004$^{\#}$ (12.7%)	0.18 ± 0.005* (30.7%)	0.16 ± 0.006* (50.0%)	0.15 ± 0.007* (56.0%)
IV	EAESC (250 mg/kg) (i.p)	0.148 ± 0.004^{ns} (7.5%)	0.18 ± 0.003* (201%)	0.148 ± 0.004* (43%)	0.132 ± 0.004* (58.7%)	0.11 ± 0.004* (68.23%)
	F- Value	4.533	10.222	48.500	152.167	222.5
	P- Value	0.018	0.001	0.000	0.000	0.000

Values expressed as ml, are mean ± SEM from 6 animals in each group, (%) inhibition shown in parenthesis comparison groups II, III,VI as group-I.# – significantly different at P < 0.05;
@ – significantly different at P < 0.01; * - significantly different at p<0.001; ns – Not significant
MESC – Methanolic Extract of *Smilax chinensis*; EAESC – Ethyl Acetate Extract of *Smilax chinensis.*

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Table.2. Effect of *Smilax Chinensis* extract (400 mg/kg i.p) on carrageenan induced Paw edema.

Group	Treatment	Edema Volume (ml)				
		1 hr	2 hr	3hr	4hr	5hr
I	Control 4% Tween 80,(10ml/kg)(i.p)	0.16 ± 0.007	0.22 ± 0.007	0.26 ± 0.007	0.32 ± 0.007	0.34 ± 0.007
II	Standard Indomethacin 20mg/kg (i.p)	0.14 ± 0.007$^{\#}$ (12.5%)	0.17 ± 0.012$^{\#}$ (22.73%)	0.14 ± 0.012* (46.15%)	0.10 ± 0.012* (68.75%)	0.06 ± 0.012* (82.35%)
III	MESC (400 mg/kg) (i.p)	0.15 ± 0.004^{ns} (6.25%)	0.18 ± 0.006$^{@}$ (18.18%)	0.16 ± 0.007* (38.46%)	0.14 ± 0.006* (56.25%)	0.13 ± 0.005* (56.0%)
IV	EAESC (400 mg/kg) (i.p)	0.144 ± 0.007^{ns} (7.5%)	0.17 ± 0.005$^{@}$ (201%)	0.14 ± 0.001* (43%)	0.12 ± 0.004* (58.7%)	0.09 ± 0.005* (73.53%)
	F- Value	0.953	7.540	40.729	171.419	254.111
	P- Value	0.438	0.002	0.000	0.000	0.000

Values expressed as ml, are mean ± SEM from 6 animals in each group, (%) inhibition shown in parenthesis comparison groups II, III,VI as group-I. # – significantly different at P < 0.05;
@ – significantly different at P < 0.01;*– significantly different at p<0.001; ns – Not significant
MESC – Methanolic Extract of *Smilax chinensis*; EAESC – Ethyl Acetate Extract of *Smilax chinensis.*

Table.3.The effect of *Smilax chinensis* extract (250 mg/kg i.p) on hot plate reaction time.

Group	Treatment	Time in Min				
		0	30	60	120	150
I	Control 4%, Tween 80, (10ml/kg)(i.p)	4 ± 0.28	3.8 ±0.178	3.6 ± 0.22	4 ± 0.28	4.54 ± 0.3
II	Standard Pentazocine 20mg/kg (i.p)	4.2 ± 0.26^{ns}	8.53 ± 0.18*	14 ± 0.16*	15 ± 0.13*	15 ± 0.13*
III	MESC (250 g/kg) (i.p)	3.4 ± 0.21^{ns}	6.7 ± 0.11*	7.3 ± 0.08*	6.94 ± 0.16*	6.9 ± 0.14*
IV	EAESC (250 mg/kg) (i.p)	3.4 ± 0.21^{ns}	7.2 ± 0.04*	8.3 ± 0.11*	7.9 ± 0.15*	7.34 ± 0.05*
	F- Value	1.108	154.297	321.109	329.721	412.087
	P- Value	0.375	0.000	0.000	0.000	0.000

Values, expressed as ml, are mean ± SEM from 6 animals in each group, % inhibition shown in parenthesis comparison groups II, III, VI as group I,# – significantly different at P < 0.05;
@ – significantly different at P < 0.01;*– significantly different at p<0.001; ns – Not significant
MESC – Methanolic Extract of *Smilax chinensis*; EAESC – Ethyl Acetate Extract of *Smilax Chinensis.*

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Table.4.The effect of *Smilax Chinensis* extract (400 mg/kg i.p) on hot plate reaction time.

Group	Treatment	Time in Min				
		0	30	60	120	150
I	Control 4% Tween 80, (10ml/kg) (i.p)	4 ± 0.28	3.8 ± 0.178	3.6 ± 0.22	4 ± 0.28	4.54 ± 0.3
II	Standard Pentazocine 5 mg/kg (i.p)	4.2 ± 0.26 ns	8.53 ± 0.18*	14 ± 0.16*	15 ± 0.13*	15 ± 0.13*
III	MESC (400 mg/kg) (i.p)	4.2 ± 0.33 ns	7.15 ± 0.05*	8.02 ± 0.12*	7.6 ± 0.14*	7.1 ± 0.06*
IV	EAESC (400 mg/kg) (i.p)	4.4 ± 0.2 ns	7.73 ± 0.14*	9.35 ± 0.16*	8.32 ± 0.08*	8 ± 0.12*
	F- Value	0.274	153.933	342.463	358.721	336.119
	P- Value	0.843	0.000	0.000	0.000	0.000

Values, expressed as ml, are mean ± SEM from 6 animals in each group, % inhibition shown in parenthesis comparison groups II, III, VI as group I,# – significantly different at P < 0.05;
@ – significantly different at P < 0.01;*– significantly different at p<0.001; ns – Not significant
MESC – Methanolic Extract of *Smilax chinensis*; EAESC– Ethyl Acetate Extract of *Smilax chinensis.*

DISCUSSION

The purpose of the paper was to establish the scientific bases for one of the traditional uses of smilax chinensis against inflammation and rheumatic pain. Carrageenan was widely used as a noxious agent to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity. The phlogistic agent when injected locally in to the raw paw, produced a severe inflammation reaction, which was discernible with in 30 min. carrageenan induced paw edema is a biphasic response. The first phase is mediated through the release of histamines, seratonin and kinnins where as second phase is related to the release of prostaglandin. An early phase corresponding to acute neurogenic pain, sensitive to drug that interact with the opioid system, and a late phase corresponding to inflammatory pain responses inhibited by analgesic-anti inflammatory drugs. Drugs that act primarily as central analgesics inhibit both phases while peripherally acting drugs inhibits only the late phase. The Ethyl acetate and Methanolic extract showed dose dependent anti-inflammatory activity and produced reduction of the duration of the licking in the late phase in analgesic activity, which was found to be statistically significant at higher concentration in acute carrageenan induced rat paw edema model and eddy's hot plate method. However, this activity was less potent as compared to reference drug. It has been reported that the second-phase edema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents [7].

CONCLUSION

The results obtained indicates that *Smilax chinensis* has significant anti inflammatory and analgesic activity when compare to control and mild when compare to reference drug. The result suggests the usefulness of extract of smilax chinensis in the treatment of inflammation associated diseases like arthritis.

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REFERENCES

[1] Giberti G. J Ethnopharmacol 1983; 7:321-41.

[2] Hunskaar S, Hole K. Pain 1987; 30:103-4.

[3] Winter CA Risley EA, Nuss GW. Proc Soc Exp Biol Med 1962:111544-52.

[4] Eddy NB, Leimbach D.. J Pharmacol Exp Ther 1953; 107:385-93.

[5] Olfert ED, Cross BM, Mc William AA, editors. Canadian Council of Animals Care guide to the care and use of experimental animals, Vol.1.2nd ed.1993.

[6] Litchfield JT, Wilcoxon F. J Pharmacol Ther 1949; 96-99-113.

[7] Katzung, B G., *Basic and Clinical Pharmacology*, Stanford, Connecticut, 1998, 7th edn, pp. 578–579.