AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Anti Nociceptive and Anti Inflammatory Activity of *Scheilchera oleosa*(lour) Oken Bark

M. Lakshmi Santha[1]*, P. Kanchana[1], CH Supriya[1]

1. Nirmala college of Pharmacy, Atmakuru, Mangalagiri, Guntur Dt. A.P 522 503, India.

ABSTRACT

Schleicheraoleosa is (Lour.) Oken belonging to the family Sapindaceae. An ethanolic extract of *Schleicheraoleosa* bark was investigated for its Anti-nociceptic and anti-inflammatory activities in rats and mice. Anti-nociceptic activity was evaluated by using the tail immersion, tail flick and hot plate latency assay while the anti-inflammatory test were carried out using the carrageenan induced paw oedema model in rats. Pentazocin was used for analgesic activity and Indomethacin was used for anti-inflammatory activities as standard drugs. The in tail immersion, tail flick and the hot plate latency times were increased with the increase in doses of 200, 400 & 600 mg/kg significantly. . Similarly, for the carrageenan induced paw oedema model the result show that the extracts at the doses of 200, 400 & 600 mg/kg significantly ($P < 0.001$) inhibited the paw oedema in a dose dependent manner. The present study proved that the ethanolic extract of S. oleosa has analgesic and anti-inflammatory activities property.

Keywords: Anti nociceptic activity, Anti-inflammatory activity, *Scheilcheraoleosa.*

*Corresponding Author Email: macharlasantha@gmail.com
Received 23 March 2015, Accepted 17 April 2015

Please cite this article as: Santha ML *et al*., Anti Nociceptive and Anti Inflammatory Activity of *Scheilchera oleosa*(lour.) Oken Bark. American Journal of PharmTech Research 2015.

INTRODUCTION

Inflammatory response is a complex process mediated by a variety of signaling molecules released by nerve endings, mast cells, platelets and leukocytes. Some of these molecules and their precursors (prostaglandins, nitric oxide, adenosine deaminase and myeloperoxidase) are used as markers of inflammation [1]. Currently, several anti-inflammatory agents are used to treat different types of pain associated with inflammation. Schleichera is a monotypic genus of plants in the family, Sapindaceae. S. oleosa is a tree and commonly known as Kusum that occurs in the Indian subcontinent and Southeast Asia. The oil extracted from the seed, called 'kusum oil' is used for cooking and lighting purpose, cure of itching, acne, burns, other skin troubles, rheumatism (external massage), hair dressing and promoting hair growth [2]. The bark is used as an astringent and against skin inflammations, ulcers, itching, acne and other skin infections [3]. It is generally used as an analgesic, antibiotic and against dysentery [4]. Various studies were also done to find out the various constituents of S.oleosa phytochemical studies have shown that its bark contains lupeol, lupeol acetate, betulin, betulinic acid, beta-sitosterol, and scopoletin[5]. From derivatives of lupane series, betulin and betulinic acid are the most effective compounds in skin inflammation. Betulinic acid shows significant anti-inflammatory activity against rat paw oedema induced by carrageenan and serotonin, which is comparable to the standard anti-inflammatory agents[6]. Betulin and betulinic acid inhibited phospholipase A_2 and showed the anti-inflammatory activity [7].

MATERIALS AND METHOD

Collection of Plant material

The stem bark powder of the plant was procured by K. Madhava Chetty, Assistant Professor, Dept. of botany, Sri Venkateswara University, Tirupati and authenticated by Prof. Dr.Vatsavaya S. Raju, M.Sc., Ph.D., D.A.S., FBS., FIAT from the Dept. Of Botany, Kakatiya University, Warangal, AP. A voucher specimen (Voucher No. 1900)has been deposited at the Herbarium of Dept. of Botany, Kakatiya University, Warangal, and AP.

Preparation of Bark Extract

The coarse powder was extracted with ethanol using cold percolation method.

Cold percolation method:

A known amount of the dried material (5gm/50mL) was soaked in ethanol and kept for occasional shaking nearly 48hrs using percolator. This was followed by filtration by using filtration and evaporation of excess solvent without applying heat. The obtained dried extract was stored at 4^0C [8]and obtained extract yield after dried was approximately 2.5gms for 25 Gms of bark powder.

Preliminary Phytochemical Screening

The ethanolic extract was screened for the presence of various phytochemical constituents like Flavonoids, terpenoids, steroids, tannins and phenolic compounds by using standard phytochemical tests.

Animals

Sparaguedawely rats (weighing 150-250gms & age 2-3 months) and Swiss albino mice (weighing 20-25gms & age 7-9 weeks) of either sex were used in this study. They were procured from Mahaveer Enterprises, Hyderabad. The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained at 22-27± 2^0C under 12hrs dark/light cycle. They were fed with standard rat pellet feed and hygeinity maximum comfort for animals. Animals Ethics Committee (Reg.1629/PO/a/12/CPCSEA) and approval No 002/IAEC/NCPA/M.Pharm/2013-14) for the care and use of animals and were strictly followed throughout the study.

Acute Toxicity studies

Acute oral toxicity was performed as per OECD-423 guidelines. The purpose of these studies is to know the safety & toxicity of the extract doses. For this study, Swiss albino mice of weights 20-25 g were selected & divided into 6 groups, each group consisting 6 animals. The animals were fasted overnight with free access of water *ad libitum*. The *Schleicheraoleosa (Lour.) Oken* extract was suspended in CMC and administered to all the 6 groups at doses 100, 300, 500, 1000, 1500 and 2000 mg/kg doses orally. The animals were closely observed for 24hr for any behavioural, physical changes and mortality. Doses were fixed based on acute toxicity studies.

Anti nocicepticactivity

a) Tail immersion method

Male Swiss Albino mice (20-25gms of body weight & 7-9 weeks of age) were used. Mice were divided in four groups and each group consists of six animals. Animals were fasted for 18hrs with free access of water *ad libitum*. Group-I is treated with 1% CMC orally and served as control. Group-II treated with Pentazocin (20mg/Kg i.p.) and served as a standard. Group-III, IV and V were administered with test doses of SOEE (200mg/Kg, 400mg/kg and 600mg/Kg,) respectively. Animals were kept in suitable restrainers with tail extended out and was markedup to 1-2 cm. The tail immersed into water bath thermo-statistically maintained at 55±1^0C. The tail withdrawal time from the hot water was noted as reaction time. The basal reaction time was measured initially and then the reactions were measured after regular interval of 30min up to 120min. i.e. at 30, 60, 90 and 120min respectively. The flick response was calculated and compared with control group.

b) HAFFNER's tail clip method

The reaction time was noted after applying an artery clip to the tail root of mice. Male mice with a weight between 20 to 25 g & age 7-9 weeks were used. . Mice were divided in four groups and each group consists of six animals. To the group-I vehicle (1% CMC oral) was administered and served as control, group-II received Pentazocine (20 mg/kg, i.p.) and served as a standard and III, IV, V test groups treated with SOEE (200, and 400 mg/kg, 600 mg/kg oral). The doses were administered 30min prior testing. An artery clip was applied to the tail root (approximately 1 cm from the body) to induce pain. The animal responds quickly to this noxious stimulus by biting the clip or the tail at the clipped area. The time between onset of stimulation and response was measured by a stop-watch in 1/10 seconds increments [9].

c) Hot plate method:

Swiss albino mice 20-25 body weight and 7-9 weeks age in five groups of six animals in each group were treated with vehicle (1% CMC) served as a control, standard drug Pentazocine (20mg/kg, i.p.) served as a standard and SOEE (200, 400 and 600 mg/kg, p.o.) served as a test groups. All doses were administered 30min prior testing. Animals were placed on a hot plate maintained at a temperature of 55 ± 0.5^0C [10]. The latency to lick the paw or jump from the hot plate was noted as the reaction time. The reaction time was noted at intervals of 30, 60, 90 and 120 min. The cut off time was considered as 15 s. The results were tabulated in Table 4.

Anti-inflammatory activity

d) Carrageenan induced paw oedema method

Sprague Dawley rats (weight 180-200gms and age 2-3 months) of either sex were divided into 5 groups. Acute inflammation was produced by sub plantar injection of 0.1mL of 1% suspension of carrageenan with 2% gum acacia in normal saline, in the left hind paw of rats, one hour after oral administration of extract doses (200, 400mg/Kg and 600mg/kg) each and Indomethacin 10mg/kg body wt. was administered by oral route. The paw volume was measured plethysmometrically (Digital volume meter) at 0, 0.5, 1, 2, and 3hr after the carrageenan injection. The difference between '0' readings and readings after 30, 60, 120 and 180 min respectively were taken as the volume of edema[11]. Percentage inhibition of edema was calculated.

RESULTS AND DISCUSSION

This study is the first report related to analgesic and anti-inflammatory activity of S. oleosa bark ethonolic extract. The analgesic activity was evaluated through tail immersion, tail clip, and Hot plate assays in mice, whereas anti-inflammatory activity was performed through carrageenan

induced paw edema in rats. In the Tail immersion method, nociceptive pain is considered as withdrawal of tail from hot water. The bark of SOEE illustrated significant (***P<0.001 and **P<0.01) increase in reaction time at the doses (200 400 and 600 mg/Kg p.o.) compared to normal group. Maximum analgesic effect of extract and standard were observed at 90 min. At 90 min standard Pentazocin (20mg/Kg), showed reaction time 6.40sec, whereas extract doses showed 5.76s, and 7.21s sec. in that order compared to control which was 4.21sec. The actual standard withdrawal time of untreated animals is between 1 and 5.5s. A withdrawal time of more than 6sec may regard as a positive response to analgesic activity which observed with all doses of SOEE [12]. Tail immersion method usually may act through opiod receptor. Whereas the Tail Clip model also employed for analgesic response which was noted as the biting the clip or the tail near the clipped area. The percent increase reaction time of the standard Pentazocin (20mg/Kg) and SOEE doses were (200,400 and 600mg/Kgp.o.) 146%, 108% and 193%, respectively. The results of the test and standard doses were given significant (***P<0.001) activity. Hence SOEE proved analgesic response compared to control. Hot plate method is depend on the sensitivity of mice paw to heat without damaging the skin which is observed as jumping, paw licking and paw withdrawal. The ethanolic extract of the *S.oleosa* significantly (***P<0.001, **P<0.01) increased the reaction time in mice and the percentage protection is nearly same to the corresponding doses. At 120 min of study, the extract increased the reaction time of heat sensation 7.44% and 50.51% at the doses (200, 400 and 600mg/Kg p.o) SOEE respectively at the same time the standard drug was 22.28%. The extract exhibited a dose dependent increase in reaction time compared with control. The hot plate method elucidates peripheral mediated effects [13]. Based on the results it shows SOEE interferes with peripheral pain mechanisms. The carrageenan test was selected because of its sensitivity in defecting orally active anti-inflammatory agents particularly in the acute phase of inflammation [14]. Standard drug (Indomethacin 10mg/Kg) diminished the paw oedema. The pharmacological evaluation of *S.oleosa* doses showed reduction in carrageenan induced paw oedema when compared with carrageenan injected control group dose dependently. The percentage decrease in paw volume at 180 min after administration of SOEE doses (200 and 400mg/Kg *p.o*) were 19.8% and 38.5% respectively and the standard dose decreased paw volume by 25%. The development of edema in the rat paw after injection of carrageen was a biphasic event. The initial phase of the edema is due to the release of histamine and serotonin and it is maintained during the plateau phase by kinin like substance and the second accelerating phase of edema is due to the release of prostaglandin like substances[15]. For this reason, it was considered that *S.oleosa* formulation

inhibiting all chemical mediators of inflammation which induces the pain response in the central nervous system.

Table 1: Preliminary phytochemical Screening of SOEE:

Test for Chemical constituents	Result
Carbohydrates	Positive
Flavonoids	Positive
Terpenoids	Positive
Steroids	Positive
Tannins	Positive
Phenolic compounds	Positive

SOEE=Schleichera *oleosa* ethanolic extract.

Table 2: Effect of S.oleosa (200, 400 and 600 mg/kg) on Tail immersion method in mice

Groups	Latency to flick tail (sec)			
	At 30 min	At 60 min	At 90 min	At 120 min
1% CMC	4.09±0.16	4.12±0.23	4.21±0.24	4.05±0.12
Pentazocin 20mg/Kg(i.p)	5.3±0.46**	6.21±0.33***	6.40±0.3***	4.72±0.24
SOEE-200 mg/Kg	4.28±0.031	5.3±0.01*	5.76±0.01***	4.16±0.01
SOEE-400 mg/Kg	6.32±0.18***	6.81±0.23***	7.21±0.27***	5.98±0.18***
SOEE -600mg/Kg	6.48±0.16***	6.87±0.24***	7.27±0.28***	5.90±0.22***

n = 6.The observations are mean ± S.E.M. **P<0.01, ***P<0.001as compared to control.

Table 3: Effect of S.oleosa (200, 400 and 600mg/kg) on Tail clip method in mice

Groups	Reaction Time in sec	% increase RT
1% CMC	2.96±0.282	-
Standard	7.27±0.391***	145
SOEE-200mg/Kg	6.16±0.372***	108
SOEE-400mg/Kg	8.65±0.422***	193
SOEE-600mg/Kg	9.63±0.453***	226.5

n = 6.The observations are mean ± S.E.M. ***P<0.001as compared to control (One way ANOVA followed by Tukey's Multiple Comparison Test). SOEE= Schleichera oleosa ethanolic extract.

Table 4: Effect of S.oleosa (200, 400 and 600 mg/kg) on Hot Plate method in mice

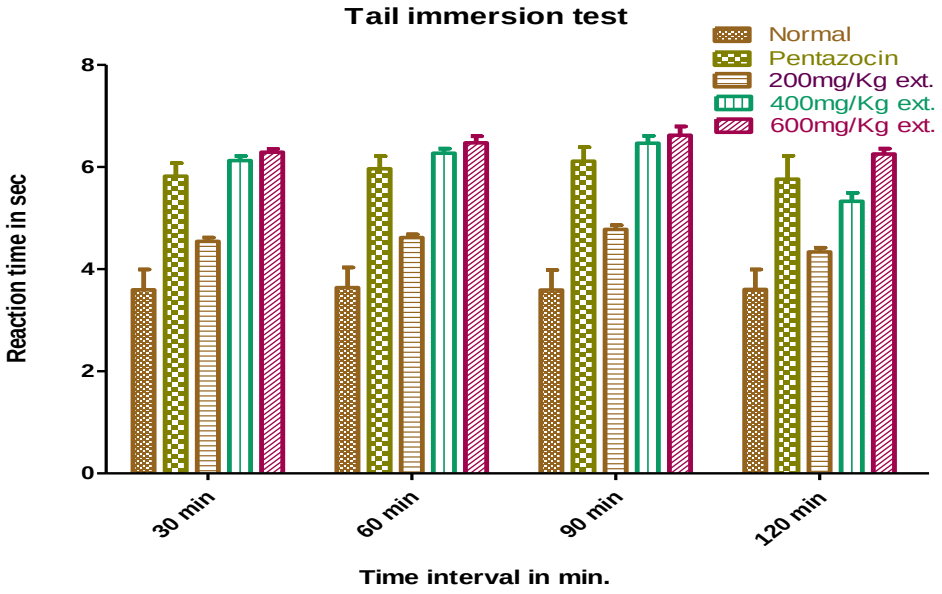
Treatment	Reaction Time (sec)				
	At 30 min	At 60 min	At 90 min	At 120 min	% increase Reaction Time at 120 min
1% CMC	3.92±0.48	3.92±0.51	3.96±0.50	3.86±0.56	-
Pentazocin 20mg/Kg	5.3±0.46*	6.2±0.33***	6.39±0.30***	4.72±0.240	22.28
SOEE - 200mg/Kg	4.65±0.303	5.06±0.68	5.71±0.31**	4.15±0.282	7.44
SOEE - 400mg/Kg	6.32±0.18***	6.81±0.23***	7.21±0.27***	5.81±0.18***	50.51
SOEE - 600mg/Kg	6.48±0.16***	6.87±0.24***	7.27±0.28***	5.93±0.22**	53.62

Anti-inflammatory Activity

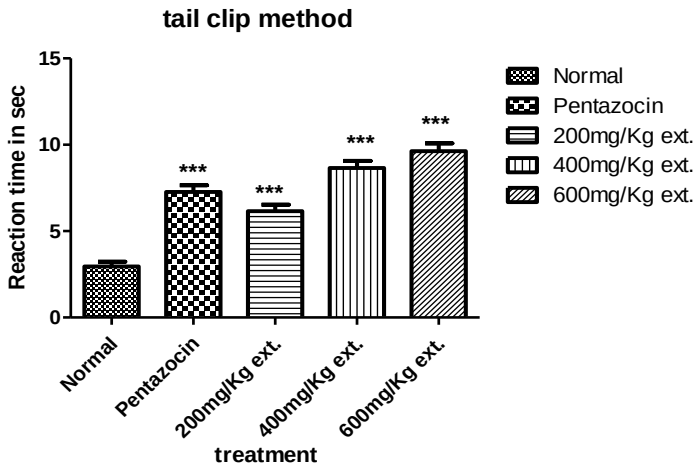
Table 5: Effect of *S.oleosa* (200, 400 and 600 mg/kg) on Carrageenan induced paw edema method in rats

Groups	Paw volume in mL					
	0 min	30 min	60 min	120 min	180 min	% decrease in paw volume Time at 180 min
1% carrageenan	0.74±0.01	0.79±0.01	1.18±0.015	1.37±0.01	0.96±0.001	-
Standard	0.65±0.01***	0.67±0.01***	0.79±0.01***	0.87±0.01***	0.72±0.004***	25
SOEE 200mg/Kg	0.72±0.01	0.75±0.01	0.88±0.01***	0.99±0.17***	0.77±0.01***	19.8
SOEE 400mg/Kg	0.66±0.01***	0.69±0.006***	0.76±0.006***	0.78±0.006***	0.59±0.006	38.5
SOEE 600mg/Kg	0.43±0.004***	0.43±0.006***	0.48±0.006***	0.51±0.007***	0.44±0.005***	54.2

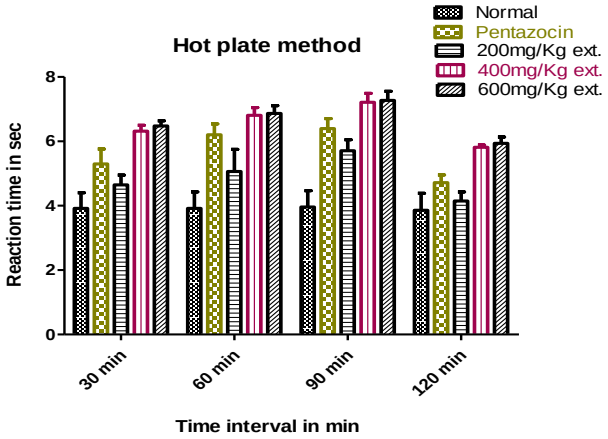
n = 6.The observations are mean ± S.E.M. ***P<0.001 as compared to control. (Two way ANOVA followed by Bonferronipost tests). SOEE= Schleichera oleosa ethanolic extract.



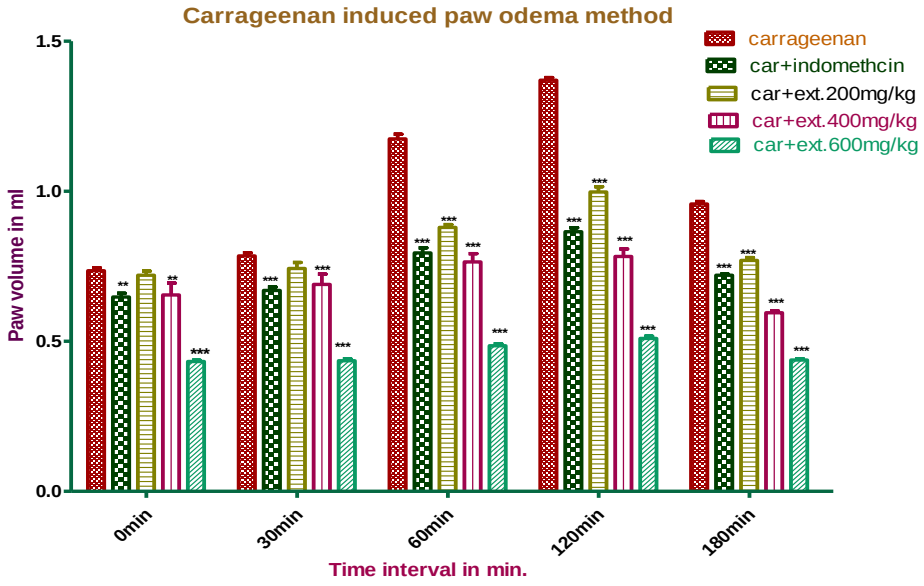
Graph 1: Tail immersion method graphical result



Graph 2. Tail clip method Graphical Result



Graph 3: Hot Plate method Graphical Result



Graph 4: Carrageenan induced paw oedema Graphical result

CONCLUSION

In conclusion, the present study proves that SOEE possess analgesic and anti-inflammatory effects, comparable to those observed with standard drugs. The mechanism of action might be associated with the inhibition of prostaglandins synthesis as observed for most NSAIDs. Further isolation of active constituents and investigation of SOEE is required to study the detailed mechanism of action with different pain and inflammation models in relation to prostaglandin synthesis. My study shows that SOEE has potential to be developed as a new product for pain & inflammation management.

REFERENCES

1. Kalkan A, Bulut V, Erel O, Avci S, Bingol NK. Adenosine deaminase and guanosine deaminase activities in sera of patients with viral hepatitis. Mem Inst Oswaldo Cruz 1999; 94, 383–6.
2. Palanuvej C, Vipunngeun N. Fatty acid constituents of Schleichera oleosa (Lour) Oken seed oil. J Health Res. 2008; 22:203-212.
3. Iwasa S. FaridahHanum I, van der Maesen LJG, Schleichera oleosa (Lour.) Oken. In: eds. Plant Resources of South-east Asia No. 11. Auxiliary Plants. Bogor Indonesia: Prosea Foundation;1997:227-229
4. Dan S, Dan SS. Phytochemical study of Adnsoniadigitata, Coccoloba excoriates Psychotriaadenophylla, and Schleichera Oleosa. Fitoterapia. 1986; 57:445-446.
5. Rout SD, Panda T, Mishra N. Ethno-medical plants used to cure different diseases by tribal of Mayurbhanj district of North Orissa. Ethno-med. 2009; 3:27-36.
6. Mukarjee PK, Saha K, Das J, Pal M, Saha BP, Studies on the anti-inflammatory activity if rhizomes of Nelumbonucifera.Planta Med. 1997;63, 367-369.
7. Bernard P, Scior T, Dider B, Hibert M, Berthon J. Ethnopharmacology and bioinformatic combination for leads discovery: application to phospholipase A2 inhibitors.2001; 58, 865-874.
8. Kokate CK Practical pharmacognosy 4th edition, vallabha prakashan, 1993,106
9. Vogel H. Gerhard (Ed.) 1927 Drug discovery and Evalution, Pharmacological assays, 2nd edition, Springer:
10. Turner RA. (1971) Screening Methods in Pharmacology, Academic Press. 100-113.
11. Sulaiman MR, Perimal EK, Akhtar MN, Mohamad AS, Khalid MH, Tasrip NA, et al. Ant-inflammatory effect of zerumbone on acute and chronic inflammation models in mice. Fitoterpia 2010; 81: 855-858

12. Vogel H. Gerhard (Ed.) 1927 Drug discovery and Evalution, Pharmacological assays, 2nd edition, Springer:
13. Vongtau HO, Abbah J, Ngazal IE, Kunle OF, Chindo BA, Otsapa PB, Gamaniel KS. Anti-nociceptive and anti-inflammatory activity of methanolic extract of Perinaripolyandra stems bark in rats and mice. J.Ethnopharmacol. 2004; 90: 115-121.
14. Dirosa M, Giroud JP, Willoughby DA. Studies of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J. Pathol. 1971; 104: 15-29.
15. Chauhan O., Godhwani J.L., Khanna N.K. and Pendse V.K., Anti-inflammatory activity of Muktashukt ibhasma, Indian J. Exp. Biol., 1998, 36, 985-989.