Research Article

Anti-inflammatory and Anti-arthritic Activity of *Scheilchera oleosa* (Lour.) Oken Bark

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ABSTRACT

Schleichera oleosa is (Lour.) Oken belonging to the family Sapindaceae. The anti-inflammatory and anti-arthritic effect of ethanolic extract of bark of *Scheilchera oleosa* was evaluated in various experimental models in rats and mice. Anti-inflammatory activity was studied by Acetic acid induced writhings, Cotton pellet granuloma and Antiarthritic activity was accessed in Freund's adjuvant induced paw oedema in rats (n=6) by SOEE (in doses of 200 mg/kg, 400mg/kg and 600 mg/kg of body wt.). Indomethacin was used as standard drug for both the activities. SOEE of doses proved significant ($P < 0.05$,$P<0.01$, $P<0.001$) decreases in writhings in mice and also decreases in dry weight of cotton in anti-inflammatory activity models. The extract doses showed significant ($P<0.001$, $p<0.01$) reduced paw oedema in dose dependent manner in carrageenan induced rats. The results obtained from the present study showed that the ethanolic extract of *S.oleosa* has anti-inflammatory and anti arthritic activity.

Keywords: Anti inflammatory, Antiarthritic, *Scheilcheraoleosa,* Complete Freund's adjuvant emulsion (CFA).

INTRODUCTION

Inflammation is a physiological process in response to tissue damage resulting from microbial pathogen infection, chemical irritation & wounding[1]. Inflammation is a normal response to any noxious stimulus that threatens the host and may vary from localized response to a generalized one[2]. The inflammatory process protects our body from diseases by releasing cells and mediators that combat foreign substances and prevent infection[3]. Inflammatory response is a complex process mediated by variety of signalling molecules released by nerve endings, mast cells, platelets and leukocytes[4]. Basically arthritis involves the breakdown of cartilage (normally protects a joint, allowing it to move smoothly), that also absorbs shock when pressure is placed on the joint, as during walking. Without the normal amount of cartilage, the bones rub together, causing pain, swelling (inflammation), and stiffness and limited movement. The need of treatment is to reduce pain, improve function, and prevent further joint damage [5].

Rheumatoid arthritis (RA) is considered the most common chronic inflammatory autoimmune disease. Rheumatoid arthritis consists of synovial joint inflammation, leading to bone and cartilage destruction. Early aggressive treatment to manage symptoms and slow disease progression include disease-modifying antirheumatic drugs (DMARDs), adjunctive non-steroidal anti-inflammatory agents and corticosteroids, physical therapy, exercise and rest[6-9].

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[10]. The bark is used as an astringent and against skin inflammations, ulcers, itching, acne and other skin infections[11]. It is generally used as an analgesic, antibiotic and against dysentery[12]. 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[13]. 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 [14] Betulin and betulinic acid inhibited phospholipase A2 and showed the anti-inflammatory activity[15].

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

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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 [16] 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°C 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 20-25g were selected & divided into 6 groups, each group consisting 6 animals. The animals were fasted overnight with free access of water *ad libitum*. The *Schleichera oleosa (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-inflammatory activity

Acetic acid induced Wriths method

The Swiss albino mice (20-25gms body weight and 7-9 weeks age) were divided into 5 groups. SOEE (200m/kg, 400mg/kg, 600mg/kg body wt., p.o each) and Indomethacin (5mg/kg body wt., p.o.) was administered one hour prior to i.p. injection of 0.1mL of 1% acetic acid. Five minutes after the intra-peritoneal injection, the number of writings during the following 10 min was counted. Finally the percent analgesic effect was determined. The number of writhings and stretching's was recorded and the percentage was calculated [17].

The percent inhibition (% analgesic activity) was calculated by

$$\textbf{\% inhibition} = \frac{\mathbf{N-Nt}}{\mathbf{N}} \times \mathbf{100}$$

Where, N = Average number of stretching of control per group.

N^t = Average number of stretching of test per group.

Cotton Pellet Granuloma method

Procedure:

Male Sprague Dawely rats with an average weight of 180-200 g and age 2-3 months were anaesthetized with ether. The back skin was shaved and disinfected with 70% ethanol. An incision is made in the lumbar region. By a blunted forceps subcutaneous tunnels were formed and a sterilized cotton pellet weighing 15mg was placed on both sides in the scapular region. The pellets are either standardized for use in dentistry formed from raw cotton which produces a more pronounced inflammation than bleached cotton. The animals are treated for 7 days orally. Then, the animals are sacrificed, the pellets prepared and dried until the weight remains constant. The net dry weight, i.e. after subtracting the weight of the cotton pellet is determined. The average weight of the pellets of the control group as well as of the test group was calculated. The percent change of granuloma weight relative to control group was determined [18].

The percent inhibition (% anti-inflammatory activity) was calculated by

$$\% \text{ inhibition} = \frac{\mathrm{N-Nt}}{\mathrm{N}} \times 100$$

Where, N = Average number of stretching of control per group.

N^t = Average number of stretching of test per group.

Anti-arthritic activity

A. Complete Freund's adjuvant induced oedema

Sprague dawely rats of either sex of age 2-3 months, weighing 180-200 g and were divided into 6 groups. The 1^{st} group as control group and 2^{nd} one as positive control group. The 3^{rd} group received standard drug Indomethcine at a dose of 10mg/kg per oral. The 4^{th}, 5^{th} and 6^{th} groups received SOEE 200mg/Kg, 400mg/Kg and 600mg/Kg each respectively by oral route. After 30 min of 0.1mL CFA injection into the sub plantar region of left hind paw on '0' day, Standard Indomethcine (10mg/Kg p.o.) and extracts were administered orally once daily till 21^{st} day[19].

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The anti- arthritic effect of the extracts as well as standard evaluate by measuring paw volume of inject paw on 4th, 8th, 14th, and 21st day of study by using plethysmometer. The mean changes in injected paw volume with respect to initial paw volume were calculated on respective days and % inhibition of paw volume with respect control group was calculated. The changes in body weight record daily. On the day 22nd blood was withdrawn from the each the animal through retro-orbital vein puncture by anaesthetizing the animal with ether. The blood was collected into vials containing EDTA and the biochemical parameters like haemoglobin content, WBC count, and RBC analysed [20].

RESULTS AND DISCUSSION

Acetic acid induced Wriths method

Table: 1: Effect of S.oleosa (200, 400 and 600 mg/kg) on Acetic acid induced Wriths method in mice:

Groups	Treatment	No. of Wriths	% inhibition
I Positive control	1% acetic acid (i.p.)	40.2±1.558	-
II Std	1% A.a+ Indomethcin-5mg/Kg (p.o)	18.7±1.054***	53.5
III 1st dose	1% A.a+ SOEE-200mg/Kg (p.o)	30.3±0.988***	24.6
IV 2nd dose	1% A.a+ SOEE- 400mg/Kg (p.o)	14.7±1.022***	63.4
V 3rd dose	1% A.a+ SOEE-600mg/Kg (p.o)	10.8±0.600***	73.1

Pretreatment of mice with standard Indomethacin (5mg/Kg) and doses of SOEE (200, 400, and 600mg/Kg p.o) significantly (***P<0.001) reduced in acetic acid induced writhing in a dose dependent manner which are 24.6%, 63.4% and 73.1% in that doses sequence, although the standard dose was 53.5%. The number of writhing and stretching's of extract and standard doses were compared to acetic acid infused control. Acetic acid induced writhing elucidated as peripheral analgesic activity. Acetic acid causes analgesia by liberating endogenous substances and many others that excite pain at nerve endings[21]. There writhing are related to increase in the peritoneal level of prostaglandins and leukotrienes[22]. The results suggest that mechanism of action of extract may be linked to lipooxygenase and/or cyclooxygenase.

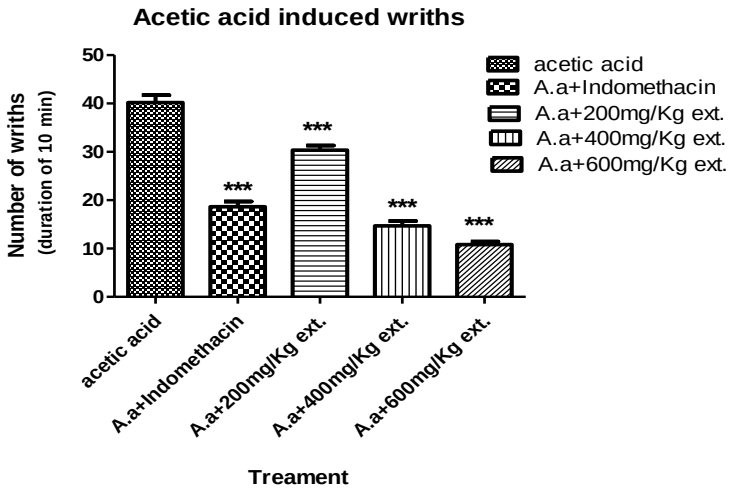

Figure 1: Acetic acid induced Wriths method Graphical result.

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* ethonolic extract.

Cotton Pellet granuloma method

The Cotton Pellet Granuloma model elicited to assess the ability of inflammatory effects of the Proliferative components of chronic inflammation [23]. Treatment *S.oleosa* (200, 400, and 600mg/Kg p.o) to rats revealed a significant (***p<0.001) inhibition in the dry weight of cotton pellet against to cotton implantation control group

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and % inhibition was found to be 35.46 %, 60.85% and 66.6% correspondingly.

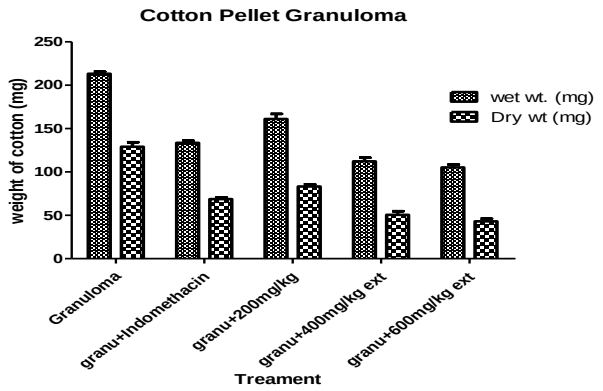

Figure 2: Cotton Pellet Granuloma Graphical Result.

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* ethonolic extract.

The reference standard (Indomethacin 10mg/Kg) inhibition was 46.75%. The *S.oleosa* decrease in the size of the granuloma may be because of the way of inhibitory action of granulocyte infiltration, preventing generation of collagen fibres and by suppression of mucopolysaccharide.

Table 2: Effect of *S.oleosa* (200, 400 and 600 mg/kg) on Cotton Pellet granuloma method in rats:

Groups	Treatment	Weight of Cotton (mg)		% inhibition of dry wt. of cotton
		Wet wt.	Dry wt.	
I Positive control	15mg cotton implant at lumbar region	213.2±2.2867	129±5.2121	---
II Std	15mg cotton +Indomethacin 10mg/Kg (p.o)	133.5±2.500***	68.75±1.7969***	46.75
III 1st dose	15mg cotton +SOEE 200mg/Kg (p.o)	161±5.9721***	83.25±2.4958***	35.46
IV 2nd dose	15mg cotton +SOEE 400mg/Kg (p.o)	112.25±4.1708* **	50.5±4.1932***	60.85
V 3rd dose	15mg cotton +SOEE 600mg/Kg (p.o)	93.5±4.1932***	43±3.0822***	66.6

Anti-arthritic Activity

In CFA model the observations such as paw volumes and body weights recorded on 1st, 4th, 8th, 14th, and 21st days after adjuvant injection. The increase in the paw volume (arthritis one of the signs enhanced) observed in CFA induced Arthritis control group which indicates primary and secondary arthritic lesion. Another observation decreased body weight also indicates the induction of arthritis in CFA control group of rats. The evaluation finished on the 21st day showed that the standard and *S.oleosa* treatment had significantly (***P<0.001, **P<0.01) reduced the paw volumes and increase in body weights in the respective treatment groups as compared with the CFA control group. The percentage inhibition of paw volumes on 21st day, standard Indomethacine (10mg/Kg, p.o.) was 40%, 200mg/kg, 400mg/Kg and 600mg/Kg doses of SOEE were 34%, 36% and 40% respectively compared against positive control.

The haematological parameters such as an increase in the WBC count, decrease in RBC count and increased Hb % were in CFA induced group also analysed in *S.oleosa* and standard treated group. Recent studies have revealed the key roles of pro-inflammatory cytokines, such as tumor necrosis factor factor-a (TNF-a), interlukin-1b (IL-1b), IL-6 and IL-8 in the pathogenesisof RA. In the present study, we showed that ethonolic extract at a dose of 200, 400 and 600mg/Kg body weight significantly inhibit the progression of the rheumatoid arthritis in treated animals. The effect of the extract was dose dependent.

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Table 3: Effect of S.oleosa (200, 400 and 600 mg/kg) on Complete Freund's adjuvant method in rats:

Groups	Treatment	Paw volume in ml				
		1st day	4th day	8th day	14th day	21st day
I positive control	1% CFA (0.1mL) Subplantar	0.17±0.011	0.26±0.013	0.35±0.011	0.35±0.006	0.25±0.006
II-Std	1% CFA +Indomethacin 10mg/Kg (p.o)	0.14±0.009	0.19±0.009***	0.23±0.009***	0.21±0.010***	0.15±0.014***
III-1st dose	1% CFA +SOEE 200mg/Kg (p.o)	0.16±0.007	0.25±0.013	0.30±0.011**	0.28±0.012***	0.19±0.011**
IV-2nd dose	1% CFA +SOEE 400mg/Kg(p.o)	0.15±0.011	0.15±0.013***	0.22±0.012***	0.18±0.008***	0.16±0.009***
V-3rd dose	1% CFA +SOEE 600mg/Kg(p.o)	0.15±0.013	0.18±0.012***	0.20±0.010***	0.17±0.011***	0.15±0.009***

Table 4: Percentage inhibition of paw volume at 21st day

Groups	Treatment	Paw Volume at 21st day	% inhibition at 21st day
I-positive control	1% CFA (0.1mL) Sub plantar	0.25±0.006	-
II-Std	1% CFA +Std10mg/Kg (p.o)	0.15±0.014***	40
III-1st dose	1% CFA +SOEE 200mg/Kg (p.o)	0.19±0.011**	34
IV-2nd dose	1% CFA +SOEE 400mg/Kg(p.o)	0.16±0.009***	36
V-3rd dose	1% CFA +SOEE 600mg/Kg(p.o)	0.15±0.009***	40

Table 5: Difference in Body weights on adjuvant-induced arthritis in rats:

Treatment	Body Weights (gms)		
	1st day	21st day	Difference in body weights
Normal	185.85 ± 4.82	197.64 ± 7.75	11.79
+ve Control	184.46 ± 4.07	166.93 ± 5.78	17.53
Std Indomethacin	177.69 ± 5.09	212.45 ± 3.95	34.76
SOEE 200mg/kg	177.48 ± 6.09	195.50 ± 4.26	18.02
SOEE 400mg/kg	189.83 ±2.15	212.72± 2.65	22.89
SOEE 600mg/kg	176.59 ±5.03	207.58± 4.86	30.99

Table 6: Effect of blood parameters on adjuvant induced arthritis in rats.

Treatment	WBC count $10^3/mm^3$	RBC count $10^6/mm^3$	Hbgm/dL
Normal	8.15±0.10	8.02 ± 0.14	14.8 ± 0.39
+ve control (CFA)	9.88 ± 0.08	5.5 ± 0.22	10.25 ± 0.48
Std Indomethacin (10mg/Kg p.o.)	8.46 ± 0.07	7.05 ± 0.08	14.75 ± 0.25
SOEE 200mg/kg (p.o.)	8.82 ± 0.07	5.91 ± 0.03	12.35 ± 0.22
SOEE 400mg/kg (p.o.)	8.76 ± 0.07	6.34 ± 0.13	13.6 ± 0.16
SOEE 600mg/kg (p.o.)	8.15 ± 0.14	7.05 ± 0.09	14.05 ± 0.22

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CONCLUSION

Based on the present study, it can be concluded that SOEE has potential anti-inflammatory effects and anti-arthritic activity 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.

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