RESEARCH ARTICLE

# INVESTIGATION OF SELECTED MEDICINAL PLANTS (*STROBILANTHES KUNTHIANUS, STROBILANTHES CUSPIDATUS*) AND MARKETED FORMULATION (SHALLAKI) FOR THEIR ANTI-INFLAMMATORY AND ANTI-OSTEOARTHRITIC ACTIVITY.

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ABSTRACT

The present study was undertaken to investigate the anti-inflammatory and anti-osteoarthritic effects of *Strobilanthes kunthianus* and *Strobilanthes cuspidatus* dried leaves alcoholic extracts (SKE and SCE) in Wistar rats. The anti-inflammatory effects of SKE and SCE was evaluated by 'Carrageenan induced rat paw edema method' and 'Cotton pellet induced granuloma formation in rats' while the anti-osteoarthritic effects of the plant extracts were investigated using iodoacetate induced cartilage damage' in rats.Shallaki (50 mg/kg) , Diclofenac (100, 10 mg/kg) and Celecoxib (100 mg/kg) were used as reference drugs for comparison.The findings of this experimental animal study indicated that *Strobilanthes kunthianus* and *Strobilanthes cuspidatus* leaves alcoholic extract possesses anti-inflammatory andanti-osteoarthritic properties thus lend pharmacological support to folkloric, ethnomedical uses of these plants in the treatment and/or management of inflammation and joint disorders.

KEYWORDS. *Anti-inflammatory, Anti-osteoarthritic, Paw edema, Cotton pellet, Cartilage damage, Strobilanthes kunthianus, Strobilanthes cuspidatus and Shallaki.*

## INTRODUCTION

The use of medicinal plants in both crude and prepared forms has increased greatly. The world health organization has estimated that 80% of the global population relies chiefly on traditional medicine for health care and there are reports that about 51% of all drug preparations in industrialized countries derive from plants, acting as sources of therapeutic agents or models for new synthetic compounds, or as raw material for semi-synthetic production of highly complex molecules.There are several herbs used in the indigenous system of medicine for the treatment of inflammation and joint disorders.

The plants *Strobilanthes kunthianus* and *Strobilanthes cuspidatus* are belongs to the Family Acanthaceae. Although there is no record of these plants being used for medicinal purposes, the tribal people of Nilgiri hills have been used in joint pains and inflammations. Therefore, this study was

undertaken to evaluate the anti-inflammatory and anti-osteoarthritic potential of extracts of these plants and will be compared with the marketed herbal formulation Shallaki which contains *Boswellia serrata* extract.

## MATERIALS & METHODS

### Plant material

The leaves of *Strobilanthes kunthianus* [1] and *Strobilanthes cuspidatus* [1] were collected, identified and authenticated by the Director, Botanical Survey of India, Coimbatore, Tamilnadu, India and sample specimens were deposited, at the Department of Pharmacology, JSS College of Pharmacy, Ootacamund, Tamilnadu, India.

### Extraction [2]

The *Leaves* of *Strobilanthes kunthianus* and *Strobilanthes cuspidatus* were air-dried in the shade and crushed to coarse powder separately. The crude ethanolic extract was obtained from the powdered botanical material separately by maceration in cold 80% ethanol with occasional agitation, for 7 days at room temperature (28-30$^0$c). The extraction was filtered and the filtrate was evaporated to dryness under reduced pressure and stored in the dark at +4$^0$c until tested. Henceforth, the ethanolic extract of *Strobilanthes kunthianus* will be called as SKE and *Strobilanthes cuspidatus* will be called as SCE.

### Qualitative phytochemical analysis [3]

The conventional chemical tests were carried out for the extract of SKE and SCE to identify the presence of various chemical constituents (Table 1).

**Table 1.Preliminary qualitative phytochemical analysis of SKE and SCE**

| TEST | SKE | SCE |
|---|---|---|
| Alkaloids | - | - |
| Carbohydrates | + | + |
| flavonoids | + | + |
| Saponins | - | - |
| Proteins & amino acids | - | - |
| Phytosterols | + | + |
| Triterpenoids | + | + |
| Fixed oils and fats | - | - |
| Tannins | + | + |
| Gums and mucilages | - | - |
| Volatile oil | - | - |

## *IN-VIVO* STUDIES

### Experimental animals

Adult Wistar albino rats (150-180 g) of either sex were procured from the laboratory animal house, J.S.S. College of pharmacy, Ootacamund, Tamilnadu, India and used in the study. The animals were kept under standard environmental conditions of room temperature (22$^0$ ±2$^0$C), relative humidity (50% ± 5%) and 12 h light and dark cycle. The animals were housed in the colony cages (either three rats or six

mice per cage) and provided feed (commercial pellets contain a balanced ration obtained from the Brook bond Lipton India limited, Bangalore) and water *ad libitum.*

All the animals were acclimatized to the laboratory environment 5 days prior to experiment. The animals were fasted overnight just prior to the experiment but allowed free access to drinking water. All the experiments were carried out in accordance with the guidelines of Institutional Animal Ethics Committee. The study was conducted after obtaining ethical committee clearance from the Institutional Animal Ethics Committee.

**I.Acute toxicity study [4]**

3 groups were formed with 10 rats in each group. All the animals were fasted over night before the test.

The treatment was given in the following manner:

Group-I - Control (Saline 0.9% Nacl; p.o)

Group-II- SKE (2000 mg/kg; p.o)

Group-III- SCE (2000 mg/kg; p.o)

In all the cases the dosing volume was fixed at 10 ml/kg body weight. The suspensions of SKE & SCE were prepared using saline (0.9% Nacl) and were administered by oral gavage.

First group was given equivolumes of saline, second group was given 2000 mg/kg body weight of freshly prepared SKE and the third group was given SCE.

The animals were observed for 0 min, 30 min, 1 hr, 2 hr, 4 hr, 6 hr and there after every day for 14 days. At the end of 14$^{th}$ day the animals were sacrificed with excessive ether anesthesia and dissected for examination of vital organs like brain, liver, kidney, lungs and heart for pathological changes.

**II.Anti – Inflammatory activity**

**1. Carrageenan induced rat paw edema method [5]**

7 groups were formed with 6 wistar rats in each group.

Test agents (SKE, SCE), shallaki and diclofenac (100mg/kg) were administered (p.o) 30 minutes before 1% carrageenan injection (0.1ml) into the sub plantar area of the right hind paw. The volumes of injected and contralateral paws were measured 1, 2 and 3 hours after induction of edema by using plethysmometer (Ugo basile, Italy).

The treatment was given in the following manner:

I Group – Control (Normal saline 10 ml/kg; p.o)

II Group – SKE (100 mg/kg; p.o)

III Group - SKE (200 mg/kg; p.o)

IV Group - SCE (100 mg/kg; p.o)

V Group - SCE (200 mg/kg; p.o)

VI Group – Shallaki (50 mg/kg; p.o)

VII Group – Diclofenac (100 mg/kg; p.o)

**Statistical analysis**

Results were analysed by one way ANOVA, followed by Turkey's multiple comparison test, 'p' value less than 0.05 were taken as significant (Table 2).

Table 2. Effect of SKE and SCE on carrageenan induced rat paw edema

| Treatment | Dose (mg/kg)., p.o | Mean increase in paw volume (mL) | | | |
|---|---|---|---|---|---|
| | | Time in hours | | | |
| | | 0 | 1 | 2 | 3 |
| Control (Saline) | 10 ml/kg | 0.27 ± 0.12 | 0.69 ± 0.07 | 0.80 ± 0.11 | 0.88 ± 0.15 |
| SKE | 100 | 0.25 ± 0.11 | 0.31 ± 0.05[c] (55.1) | 0.41 ± 0.02[c] (48.7) | 0.56 ± 0.04[a] (36.4) |
| SKE | 200 | 0.23 ± 0.07 | 0.27 ± 0.02[c] (60.8) | 0.35 ± 0.03[c] (56.2) | 0.40 ± 0.02[c] (54.5) |
| SCE | 100 | 0.25 ± 0.12 | 0.29 ± 0.08[c] (57.9) | 0.38 ± 0.03[c] (52.5) | 0.48 ± 0.02[c] (45.4) |
| SCE | 200 | 0.23 ± 0.01 | 0.27 ± 0.04[c] (60.8) | 0.33 ± 0.01[c] (58.7) | 0.37 ± 0.01[c] (57.9) |
| Shallaki | 50 | 0.23 ± 0.06 | 0.30 ± 0.04[c] (56.5) | 0.39 ± 0.04[c] (51.2) | 0.45 ± 0.02[c] (48.8) |
| Diclofenac | 100 | 0.24 ± 0.02 | 0.26 ± 0.06[c] (62.3) | 0.29 ± 0.01[c] (63.8) | 0.34 ± 0.03[c] (61.3) |

Values are Mean ± SEM (n=6)

a-P<0.05, b-P<0.01, c-P<0.001 when compared control vs. treatment groups

d-P<0.05, e-P<0.01, f-P<0.001 when compared SKE 100 vs. SKE 200

g-P<0.05, h-P<0.01, i-P<0.001 when compared SCE 100 vs. SCE 200

by one-way ANOVA followed by Turkey's multiple comparison test.

Each value in parentheses indicates the percentage inhibition rate.

**2. Cotton pellet induced granuloma formation in rats [6]**

7 groups were formed with 6 rats in each group.

A sterilized cotton pellet weighing 20 mg was introduced subcutaneously into the groin region of rats after the rats have been anaesthetized with ketamine. Following the implantation of the cotton pellet, the animals in the control, test group and reference groups were treated once daily for 4 days with saline, test agents (SKE, SCE), shallaki and diclofenac respectively. All the animals were sacrificed on the fifth day with an overdose of phenobarbitone sodium (40 mg/kg; i.p.) and the pellet surrounded by granuloma tissue was dissected out carefully and dried overnight in an oven at 60°C to a constant weight. The mean weight of the granuloma tissue formed in each group was obtained

and the percentage inhibition was determined by comparing the mean weight in the control group. [65]

The treatment was given in the following manner:

I Group – Control (Normal saline 10 ml/kg; p.o)

II Group – SKE (100 mg/kg; p.o)

III Group - SKE (200 mg/kg; p.o)

IV Group - SCE (100 mg/kg; p.o)

V Group - SCE (200 mg/kg; p.o)

VI Group – Shallaki (50 mg/kg; p.o)

VII Group – Diclofenac (10mg/kg; p.o)

**Statistical analysis**

Results were analyzed by one way ANOVA, followed by Turkey's multiple comparison test, 'p' value less than 0.05 were taken as significant (Table 3).

**Table 3. Effect of SKE and SCE on cotton pellet induced granuloma in rats**

| S.No. | Treatment | Dose (mg/kg).,p.o | Increase in weight of pellet ( mg ) | % inhibition |
|---|---|---|---|---|
| 1 | Control (Saline) | 10 ml/kg | 49.26±3.18 | - |
| 2 | SKE | 100 | 38.31±2.11 | 22.22 |
| 3 | SKE | 200 | 35.7±2.83[b] | 27.52 |
| 4 | SCE | 100 | 36.71±2.64[a] | 25.47 |
| 5 | SCE | 200 | 31.38±2.48[c] | 36.29 |
| 6 | Shallaki | 50 | 37.46±2.12[a] | 23.95 |
| 7 | Diclofenac | 10 | 27.41±1.74[c] | 44.35 |

**Values are Mean ± SEM (n=6)**

a-P<0.05, b-P<0.01, c-P<0.001 when compared control vs. treatment groups

d-P<0.05, e-P<0.01, f-P<0.001 when compared SKE 100 vs. SKE 200

g-P<0.05, h-P<0.01, i-P<0.001 when compared SCE 100 vs. SCE 200

by oneway ANOVA followed by Turkey's multiple comparison test.

Each value in parentheses indicates the percentage inhibition rate.

**III.Anti-osteoarthritis activity**

**1.Iodoacetate induced osteoarthritis [7]**

8 groups were formed with 10 rats in each group.

The rats were anaesthetized with ketamine and arthritis was induced by 25 µl of intra-articular injection of 10mg/ml concentration of iodoacetate into the left knee joint of rats. Then the rats were treated with test agents (SKE, SCE), shallaki and standard (diclofenac, celecoxib) for a period of 21 days. After 21 days the animals were sacrificed by excess dose of phenobarbitone sodium (40mg/kg,

i.p.) and left joint was immediately disarticulated and fixed in 10% buffered formalin for 24-48 h prior to capturing the image.

The treatment was given in the following manner:

I Group – Control (Normal saline 10 ml/kg; p.o)

II Group – SKE (100 mg/kg; p.o)

III Group - SKE (200 mg/kg; p.o)

IV Group - SCE (100 mg/kg; p.o)

V Group - SCE (200 mg/kg; p.o)

VI Group – Shallaki (50 mg/kg; p.o)

VII Group – Diclofenac (100mg/kg; p.o)

VIII Group - Celecoxib (100mg/kg; p.o)

**Articular cartilage lesion score**

After fixation, an image of the tibial cartilage was captured using an Optimas image analysis system. The tibial plateau was utilized for image analysis because it provided a relatively flat surface compared with the femoral condyles, allowing the image analysis camera to focus on the entire cartilage surface.

Cartilage damage was assessed using a scale of 0-4 of increasing severity [0=normal; 4=maximum severity].

**Statistical analysis**

Results were analyzed by Mann-Whitney test, 'p' value less than 0.05 were taken as significant (Table 4).

**Table 4. Effect of SKE and SCE on joint damage in the rat iodoacetate model**

| S.No. | Treatment | Dose (mg/kg).,p.o | Cartilage lesion score (Mean ± SEM) | % reduction |
|---|---|---|---|---|
| 1 | Control (Saline) | 10 ml/kg | 2.66 ± 0.21 | - |
| 2 | SKE | 100 | 2.24 ± 0.17* | 15.8 |
| 3 | SKE | 200 | 1.83 ± 0.16*† | 31.20 |
| 4 | SCE | 100 | 2.10 ± 0.14* | 21.06 |
| 5 | SCE | 200 | 1.50 ± 0.11*# | 43.60 |
| 6 | Shallaki | 50 | 2.0 ± 0.13* | 24.81 |
| 7 | Diclofenac | 100 | 2.12 ± 0.24* | 20.31 |
| 8 | Celecoxib | 100 | 1.40 ± 0.12* | 47.36 |

**Values are expressed as the mean joint damage or cartilage lesion score ± SEM from 10 animals.**

* P<0.05 when compared control vs. treatment groups

† P<0.05 when compared SKE 100 vs. SKE 200

# P<0.05 when compared SCE 100 vs. SCE 200

by using Mann-Whitney test.

**RESULTS & DISCUSSION**

The air-dried and finely ground leaves of *Strobilanthes kunthianus* and *Strobilanthes cuspidatus were* subjected to cold maceration with 80% ethanol, yielded 11.4% W/W and 10.8% W/W respectively.

Phytochemical analysis in both the plant extracts showed similar phytoconstituents viz. carbohydrates, triterepenoids, phytosterols, flavonoids, and tannins. Several phytoconstituents like flavonoids [8], phytosterols [9], triterpenoids [10] and tannins [11] are known to have anti-inflammatory and anti-arthritic properties.

In acute toxicity studies, SKE and SCE did not showed any toxic symptoms or caused death of rats even after 14 days at 2000mg/kg of the extracts.

Carrageenan induced rat paw edema has a significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation. Carrageenan induced inflammatory process is believed to be biphasic. The initial phase seen at the first hour is attributed to the release of histamine and serotonin. The second accelerating phase of swelling is due to the release of prostaglandin, bradykinin and lysozyme. So, the extracts SKE and SCE probably acts by the inhibition of the release of the inflammatory mediators specially prostaglandin, bradykinin and lysozyme. The result obtained from the carrageenan induced paw edema was markedly inhibited by the oral administration of the extracts SKE and SCE, thus indicating that the extracts can inhibit acute inflammatory process and that the extracts are orally active. This is because carrageenan induced paw edema is an acute model of inflammation and has been reported to be active in detecting orally active anti-inflammatory drugs.

The extracts were further evaluated by cotton pellet induced granuloma formation to understand their potential in the chronic inflammatory phase. Cotton pellet granuloma is the index of proliferative phase of inflammation. Chronic inflammation involves proliferation of macrophages and neutrophils which are modulators of granuloma formation. Therefore, decrease in granuloma weight indicates the suppression of proliferative phase. The results of the present study indicate the extracts SKE and SCE effectively suppressed granuloma formation induced by cotton pellets. These results suggest that the extracts SKE and SCE inhibited the acute and chronic phase of inflammation in a dose dependent manner.

The injection of iodoacetate into the knees of rats provides a model where lesions resembling some aspects of human osteoarthritis can be quickly produced and has been suggested as a model for the study of chondroprotective drugs. The injection of iodoacetate induces the loss of cartilage proteoglycan. Proteoglycan loss was followed by a severe thinning of the cartilage and the development of lesions in the region of the subchondral bone and calcified cartilage consisting of fibrous tissue, infiltrating mononuclear cells and blood vessels. The extracts SKE and SCE significantly reduced the cartilage damage in a dose dependent manner. Both the extracts SKE (200 mg/kg) and SCE (200 mg/kg) showed better response than the herbal positive control. The inhibition of joint damage in this model by the extracts SKE and SCE supports the further evaluation of the therapeutic potential in human osteoarthritis.

The ethanolic extracts of *S.kunthianus* and *S.cuspidatus* showed anti-inflammatory and anti-osteoarthritis effect which may be due to the presence of phytosetrols.

The extracts (SKE and SCE) have anti-inflammatory and anti-osteoarthritis potential may be due to the presence of multiple phytoconstituents such as flavonoids, phytosterols, triterpenoids and tannins.

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