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Research Article

IN-VITRO ANTI-INFLAMMATORY AND ANTI-OSTEOARTHRITIC ACTIVITIES OF *STROBILANTHES KUNTHIANUS* AND *STROBILANTHES CUSPIDATUS*

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ABSTRACT

In-vitro anti-inflammatory and anti-osteoarthritic effects of ethanolic extracts of Strobilanthes *kunthianus* and *Strobilanthes cuspidatus* was studied using 'Human RBC membrane stabilization method' and 'Rabbit cartilage explants culture method' respectively. Shallaki (50 μg/ml), Diclofenac (50 μg/ml) and Celecoxib (50 μg/ml) were used as reference drugs for comparison. The results revealed that both SKE and SCE have anti-inflammatory and anti-osteoarthritic activity. Moreover, the extracts showed equipotent activity to Diclofenac and higher activity than Shallaki.

Keywords: Anti-inflammatory, Anti-osteoarthritic, *Strobilanthes kunthianus, Strobilanthes cuspidatus*.

INTRODUCTION

Herbal medicine is the use of plants and plant extracts to treat disease, something mankind has always done. Herbal medicine exists in many local varieties depending on the regional flora. Many modern drugs were originally extracted from plant sources, even if they are now made synthetically, and many other drugs are descended from plant substances. For instance, aspirin, the original non-steroidal anti-inflammatory drug (NSAID) and 'grandfather' of a large family of such drugs, was originally extracted from willow bark. Some of the most promising herbs for arthritis, all of which are backed by some research, are devil's claw (made from a plant which grows in Namibia), *Boswellia* (from the frankincense tree) and rosehip. Generally speaking they are safe. The plants *Strobilanthes kunthianus* and *Strobilanthes cuspidatus* are belongs to the Family Acanthaceae. The tribal people of Nilgiri hills have been used these plants in joint pains and inflammations but there were no scientific records. Hence, this study was undertaken to investigate the *in-vitro* anti-inflammatory and anti-osteoarthritic activities of these plants extracts and will be compared with the marketed herbal formulation Shallaki which contains *Boswellia serrata* extract.

MATERIALS AND METHODS

Plant material

The *Strobilanthes kunthianus*[1] and *Strobilanthes cuspidatus*[1] leaves 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 *Strobilanthes kunthianus* and *Strobilanthes cuspidatus* leaves were air-dried in the shade separately and crushed to coarse powder . 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 preliminary chemical tests were carried out for the extract of SKE and SCE to identify the presence of various phytoconstituents.

IN-VITRO STUDIES

I. Anti – Inflammatory activity

Human RBC membrane stabilization method[4]

Human Red Blood Cells membrane stabilization was used as a method to study the anti-inflammatory activity. Blood was collected from a healthy volunteer who was not taken any NSAIDs for 2 weeks prior to the experiment; and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of SKE, SCE & Shallaki were prepared (10, 50 and 100µg/mL), and to each concentration, 1mL of phosphate buffer, 2 mL of hypo saline and 0.5 mL of HRBC suspension were added and incubated the mixture at 56^0 c for 30 min.The tubes were cooled under running water for 20 min, the mixture was centrifuged. The hemoglobin content in the supernatant solution was estimated spectrophotometrically at 560nm. Diclofenac (50 µg /mL) was used as a reference standard and distilled water as control in this study. The percentage of HRBC membrane stabilization was calculated (by assuming the haemolysis produced in distilled water as 100%) using the formula:

$$\% \text{ protection} = \frac{100 - \text{optical density of drug treated sample}}{\text{Optical density of control}} \times 100$$

Statistical analysis

Results were analyzed by student's t-test, 'p' value less than 0.05 were taken as significant (Table 1).

Table 1: Effect of SKE and SCE on human RBC Membrane stabilization

S. No.	Treatment	Concentration (µg/ml)	Activity (Prevention of lysis %) (Mean ± SD)
1	SKE	10	43.18 ± 0.08
		50	50.16 ± 0.05
		100	41.10 ± 1.14
2	SCE	10	48.11 ± 0.09
		50	55.24 ± 0.02
		100	46.35 ± 0.04
3	Shallaki	50	42.16 ± 0.09
4	Diclofenac	50	51.11 ± 0.07

Values are mean ± SD (n=6 trials per group)

II. Anti-Osteoarthritis studies

Rabbit cartilage explants culture method [5]

Articular cartilages from hock joints of 5-week-old rabbit were removed immediately after the animal was sacrificed. The care and handling of the animal was in accordance with the guidelines of Institutional Animal Ethics Committee. After the articular surfaces were exposed surgically under sterile conditions, approximately 200-220 mg of articular surfaces per joint were dissected and submerged into complete medium (DMEM, supplemented with heat inactivated 5% FBS; penicillin 100U/ml; streptomycin 100 µg/ml). They were then rinsed several times with the complete medium and incubated for 1 to 2 days at 37^{0}C in a humidified 5% CO_2/95% air incubator for stabilization. The complete medium was replaced with a basal medium (DMEM, supplemented with heat-inactivated 1% FBS, 10mM HEPES, and penicillin 100U/ml, streptomycin 100 µg/ml). Approximately 50 to 60 mg cartilage pieces were placed in 24-well plates and treated with 10,50,100 and 200 µg/ml concentrations of SCE and SKE, shallaki (50 µg/ml), diclofenac (50 µg/ml) and celecoxib (50 µg/ml). After pretreatment for 1 h, 5 ng/mL of rhIL-1α was added to the culture medium and further incubated at 37^{0}C in a humidified 5% CO2/95% air incubator. The culture medium was collected 60 h later and stored at -20^{0}C until assay.

Measurements of glycosaminoglycans (GAG)

The amount of sulphated GAGs in the medium at the end of reaction reflecting the amount of proteoglycan (PG) degradation was determined through 1,9-dimethyl-methylene blue method using a commercially available kit (The Blyscan proteoglycan & glycosaminoglycan assay kit, Biocolor Ltd.,). The amount of glycosaminoglycan release was estimated spectrophotometrically at 656 nm.

Statistical analysis

Results were analyzed by student's t-test, 'p' value less than 0.05 were taken as significant (Table 2).

Table 2: Effect of SKE and SCE on rhIL-1α induced degradation of proteoglycan in rabbit cartilage explants culture

S. No.	Treatment	Concentration (µg/ml)	Amount of glycosaminoglycan released (µg/ml) (mean ± SD)	% inhibition of proteoglycan degradation
1	Vehicle	-	0.31 ± 0.04	-
2	Control	-	1.52 ± 0.06	-
3	SKE	10	1.03 ± 0.09^b	32.2
		50	0.65 ± 0.02^c	57.2
		100	0.44 ± 0.01^c	71.0
		200	0.73 ± 0.06^c	51.9
4	SCE	10	0.93 ± 0.05^c	38.8
		50	0.56 ± 0.01^c	63.1
		100	0.33 ± 0.03^c	78.3
		200	0.67 ± 0.08^c	55.9
5	Shallaki	50	0.72 ± 0.03^c	52.6
6	Diclofenac	50	0.83 ± 0.07^c	45.4
7	Celecoxib	50	0.29 ± 0.03^c	80.9

Values are Mean ± SD (n=6 trials per group)
a-P<0.05, b-P<0.01, c-P<0.001 when compared to control by student't' test

RESULTS AND DISCUSSION

Phytochemical analysis in both the plant extracts showed similar phytoconstituents viz. carbohydrates, triterepenoids, phytosterols, flavonoids, and tannins.

Several phytoconstituents like flavonoids [6], phytosterols [7], triterpenoids [8] and tannins [9] are known to have anti-inflammatory and anti-arthritic properties.

The membrane stabilizing activity of two ethanolic extracts SKE and SCE at

concentrations 10, 50, and 100 µg/ml were studied on heat induced lysis of human red blood cell membrane (HRBC membrane). The extracts SKE and SCE at a concentration of 50 µg/ml shown highest protection (50.16% and 55.24% respectively) and the effect was equipotent to diclofenac – 50 mg/kg (51.11%) and more than Shallaki (42.16%).

The protective activity of two ethanolic extracts SKE and SCE at concentrations 10, 50, 100, and 200 µg/ml on rhIL-1α induced degradation of proteoglycan in rabbit cartilage explants culture was studied and the percentage inhibition of proteoglycan degradation was calculated. Treatment with SKE and SCE at a concentration of 100 µg/ml shown significant ($p<0.05$) protective effect (71% and 78.3% respectively) in inhibiting the proteoglycan degradation and the effect was equipotent to celecoxib – 50 µg/ml (80.9%). Moreover, the activity of diclofenac – 50 µg/ml (45.4%) and shallaki - 50 µg/ml (52.6%) were found to be lesser when compared to SKE (100 µg/ml) and SCE (100 µg/ml).

CONCLUSION

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

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