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Short Communication

Anti-inflammatory activity of methanolic extract of *Stephania wightii* (Arn) Dunn- an endemic medicinal plant

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The methanolic extract of aerial parts and tuber of *Stephania wightii* has been evaluated for their *in vitro* and *in vivo* anti-inflammatory activity using human red blood cell membrane stabilization method and carragennan induced paw oedema in mice model. The methanolic extract of *S. wightii* tuber part showed 75% protection of human red blood cell membrane. The result indicated that the methanolic extract of tuber at the dose of 100mg/kg.body wt., showed a maximum inhibition of paw oedema at 75.3% as compared to the reference drug, indomethacin. The methanolic extract of aerial part with a similar dose produced 63% of inhibition. The methanolic extract of tuber of *S. wightii* has showed maximum anti-inflammatory activity.

Keywords: *Stephania wightii,* methanol extract, HRBC and carragennan induced paw oedema.

Introduction

Inflammation or flogose is a reaction of the tissue blood vessels against aggressor agent characterized by access of liquids and of cells to interstice (Lope *et al.,* 1987). The inflammatory reaction is characterized by blush, heat, tumor, pain and lost function (Dassoler *et al.,* 2004). The inflammatory agent acts in the cell membranes inducing the activation of phospholipase A2 and consequently, liberates arachidonic acid and metabolites. According to Dassoler *et al.,* (2004) the inflammatory mediators such as cytokine, histamine, serotonin, leukotrienes and prostaglandin increase the vascular permeability to all on the migration leukocytes cells to act on the site of inflamed tissue. Any interruption of this sequence of events results in the reduction of the liberation of the mediators causing the microcirculation to come back to normal homodynamic state (Lope *et al.,* 1987). The non-steroidal anti-inflammatory drugs (NSAIDs) are one of the categories of drugs most frequently used by people. However, these drugs caused adverse gastric reactions, inhibit renal function, reduce the efficacy of the diuretics and retard the angiotensin converting enzyme inhibitors (Gaddi *et al.,* 2004). Natural products have long history as an important source of anti-inflammatory drugs. Recent research approaches used to analyze the anti-inflammatory potential of plant and plant derived compounds.

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The genus *Stephania* belongs to family Menispermaceae, a large family of about 65 genera and 350 species, distributed in warmer parts of the world. *Stephania wightii* (Arn) Dunn is native to Africa, India, South-East Asia and the northern and eastern parts of Australia. They are herbaceous perennial vines growing to around four meters tall, with a large, woody caudex. The leaves are arranged spirally on the stem, and peltate, with the leaf petiole attached near the centre of the leaf. The name *Stephania* means "a crown". This refers to the anthers being arranged in a crown like manner. It is locally called as "Koloukone" by kanikar tribals. In traditional medicine, *S. wightii* has been used to treat a wide variety of ailments (Gaur, 1999).

Materials and Method

A fresh plant specimen of *Stephania wightii* for the proposed study was collected from Western Ghats, Waynad, Kerala. The authenticity of the freshly collected plant was confirmed by comparing their morphological characters with the description mentioned in the different standard texts and floras. The identification of the plant was also confirmed by C. Kalidass, Taxonomist, Botanical Survey of India, Coimbatore.

Extraction

Air dried and coarsely powdered *Stephania wightii,* aerial parts and tuber separately were taken. Extraction was carried out by cold extraction method using methanol. The extract was then concentrated to dryness under reduced pressure and controlled temperature. Final traces of methanol were removed and they were preserved in a refrigerator (Khandelwal, 2002).

Animals

Male swiss albino mice weighing 20-25g were procured from Kerala agricultural University, Animal house, Trissur. All the animals were kept in standard polypropylene cages and maintained under standard conditions: temperature (24±1°C), relative humidity (45-55%) and 12:12 light: dark cycle. The animals were allowed to acclimatize to laboratory conditions 48 hrs before the start of the experiment. Groups of 6 mice were used in all sets of experiments. Animals were provided with standard rodent pellet diet and the food was withdrawn 18-24 h before the experiment though, water was allowed *ad libitum*. All the experiments were conducted after obtaining permission and performed according to ethical guidelines for the investigation of experimental pain in conscious animals (659/02/a/CPCSEA).

Acute Toxicity Study

For the pharmacological tests, the extract was suspended in double distilled water containing carboxy methyl cellulose (1% w/v, CMC). The acute toxicity was determined for the methanolic extract of *Stephania wightii* on swiss albino mice by fixed dose method. 100-1000mg/kg of methanolic extracts of aerial parts and tuber part of *Stephania wightii* was administered by oral route to mice. Mortality was observed for 3 days.

***In vitro* Anti-Inflammatory Activity**

The human red blood cell (HRBC) membrane stabilization method was used for this study. The blood was collected from healthy human volunteer who was not taken any non steroidal anti-inflammatory drugs 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 for 20 min. The packed cells were washed with Isosaline and a 10% suspension was made. Methanolic extract of aerial parts and tuber part of *S. wightii* were

prepared (100mg/ml) using with carboxy methyl cellulose and to each concentration 1ml of phosphate buffer, 2ml of hyposaline and 0.5ml off HRBC suspension were added. It is incubated at 37^{o}C for 30min and centrifuged at 3000rpm for 20min. The hemoglobin content of the supernatant solution was estimated spectrometrically at 560nm. Diclofenac (50mg/ml) was used as reference standard and a control was prepared omitting the extracts (Rajendran vadivu *et al.,* 2008).

***In vivo* Anti-Inflammatory Activity**

Carrageenan-Induced Paw Oedema in Albino Mices

Animals were divided into 5 groups comprising five animals in each group. In all groups acute inflammation was produced by sub plantar injection of 0.1ml freshly prepared 1% suspension of carrageenan in normal saline in the right hind paw of the mice and paw volume was measured plethysommetrically at 0 to 180mins after carrageenan injection. All the animals were premedicated with indomethacin (10mg/kg b.wt.) orally two hour before infection. Mean increase in paw volume was measured and percentage was calculated for all the extracts. All the extracts were subjected for acute toxicity studies and 1/10th of the LD_{50} dose was selected for pharmacological activity (Winter and Poster, 1957). Percentage inhibition of paw volume was calculated by the following formula

$$\% \text{ inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where

Vt- means increase in paw volume in mice treated with test compounds

Vc- means increase in paw volume in control group of mice

Results and Discussion

The methanolic extracts of *S. wightii* were studied for *in vitro* anti-inflammatory activity in concentration dependent manner by HRBC membrane stabilization method. The methanolic tuber extract showed high level of anti-inflammatory activity compared to the aerial part extract. The results were statistically significant ($p<0.001$) relative to control and comparable to that of the standard drug. Methanolic extract of aerial part of *S. wightii* showed 65.38% protection of HRBC hypotonic solution. The tuber part of methanolic extract showed 75% protection of HRBC in 100mg/ml concentration. All the results were compared with standard diclofenac, which showed 80.7% protection (Table 1).

The anti-inflammatory activity of aerial parts and tuber part of methanolic extracts of *S. wightii* were evaluated by carrageenan induced mice paw oedema method. The extracts were tested at 100mg/kg b.wt., dose levels. The present study elucidates 100mg/kg b.wt., methanolic extracts of *S. wightii* significantly reduced the carrageenan induced paw oedema inflammation as compared with that of the standard drug, indomethacin. This result indicated that the methanolic extract of tuber part with the dose of 100mg/kg.b.wt showed a maximum anti-inflammatory activity at 75.3% as compared to the reference drug, indomethacin. The methanolic extract of aerial part with a dose of 100mg/kg b.wt., produced 63% of inhibition (Table 2).

Similarly, the hexane, chloroform, ethyl acetate and methanol extracts of *Stephania dinklagei* were screened for anti-inflammatory activities using carrageenan, kaolin-carrageenan and formaldehyde-induced paw edema models of inflammation. The extracts showed dose dependent anti-inflammatory activity with 300mg/kg of the extracts being more potent. At 1

and 4 h, post carrageenan injection, the paw edema in the 100 and 300mg/kg hexane and ethyl acetate treated mice was significantly lower than the paw edema in the control group. Treatment of mice with 100 and 300mg/kg hexane and ethyl acetate extracts significantly suppressed the progression of edema post kaolin-carrageenan injection. The paw edema induced by formaldehyde in the 300mg/kg hexane and ethyl acetate treated groups was significantly lower than edema in the other treatment groups on day 1, 4 and 8 post formaldehyde injections (Udegbunam *et al.*, 2012).

Table 1: *In vitro* anti-inflammatory activity of methanolic extract of aerial parts and tuber of *S. wightii* by HRBC membrane stabilization method

Treatment group	Concentrations of doses	Absorbance 540nm	% of inhibition
Group I	Normal saline	0.52	--
Group II	50 (mg/kg)	0.42	80.7
Group III	100(mg/kg)	0.34	65.38
Group IV	100(mg/kg)	0.39	75.00

Group I : Control treated with normal saline; Group II: Treated with diclofenac at the dose of 50mg/kg b.wt.; Group III: Treated with methanolic aerial parts extract of *S. wightii* at the dose of 100mg/kg b.wt. Group IV: Treated with methanolic tuber extract of *S. wightii* at the dose of 100mg/kg b.wt.

Table 2: *In vivo* anti-inflammatory activity of methanolic extract of aerial parts and tuber of *S. wightii* on carrageenan induced hind paw oedema in mice

Treatment Groups	Concentrations of doses	Paw thickness (mm)				% Inhibition
		0 min	60 min	120 min	180 min	
Group I	Normal saline	5.58±0.05	7.87±0.14	10.01±0.47	12.37±0.78	--
Group II	100 (mg/kg)	5.43±0.8	5.66±0.07	5.98±0.17**	7.81±0.29	63
Group III	100 (mg/kg)	5.32±0.06	5.48±1.6*	5.71±0.48	5.85±0.09**	75.3
Group IV	10 (mg/kg)	5.27±0.50	4.94±0.07	4.68±0.15	4.62±0.48**	79.2

Value is SEM ± 5 individual observations * P < 0.05; ** P<0.01 Compared paw oedema induced control vs drug treated mice. Group I : Control mice given normal saline; Group II: Mice given methanolic aerial parts of *S. wightii* at the dose of 100mg/kg b.wt.; Group III: Mice given methanolic tuber of *S. wightii* at the dose of 100mg/kg b.wt.; Group IV: Mice given Indomethacin at the dose of 10mg/kg b.wt.

In conclusion the present study showed that the tuber part of methanolic extracts of *Stephania wightii* possessed potent anti-inflammatory properties than the aerial parts extract because the tuber part contain more amount of alkaloids. Isolation and characterization of the active constituents of both extracts of *S. wightii* will be the subject of further study and continue the search for novel anti-inflammatory drug from this plant.

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