

**Research Paper**

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**ANTI-ALLERGIC, ANTI-PRURITIC, AND ANTI-INFLAMMATORY ACTIVITIES OF  
CENTELLA ASIATICA EXTRACTS****Mathew George\*, Lincy Joseph and Ramaswamy.**

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**E-mail:** [mathewlincg@yahoo.com](mailto:mathewlincg@yahoo.com)**Abstract**

This study investigated antipruritic and anti-inflammatory effect of *Centella asiatica* extract in rats and anti-allergic *in vitro* using sheep (*Capra hircus*) serum method and compound 48/80 induced mast cell degranulation method, compared with standard drug ketotifen fumarate. In rats, extract of *Centella asiatica* administered orally was examined for anti-pruritic study and chlorpheniramine maleate was used as standard drug while carageenan paw induced inflammatory method was used for the anti-inflammatory study. The results show that the extracts of *Centella asiatica* exhibited antiallergic, anti-pruritic and anti-inflammatory activities.

**Key words:** Anti-pruritic, Anti-allergic, Anti-inflammatory, *Centella asiatica* extract.**Introduction**

Renewed interest on biological activities of medicinal plants emerged in early 1980's as the Council of Scientific and Industrial research have published the information on the screening of biological activities of many medicinal plants using experimental models (Somchic et al., 2004). Recently the use of herbal preparations in remedies for various medical conditions have been rapidly increasing especially in India. It is believed that herbal preparations are safe although the ingredients have never been vigorously substantiated. *Centella asiatica* (family-umbelliferae) is used as anti-bacterial, anti-microbial, wound healing, anti-oxidant and analgesic drug. There is a growing interest in co-relating phytochemical constituents of plants with its pharmacological activities. Scientists have started co-relating the botanical properties of plants with their pharmacological activities (Cheng and Koo, 2000).

Allergy is a genetic condition that causes the body to respond to harmless substances in the environment as though they are harmful to the body. This response produces symptoms that range from mild to life threatening episodes in susceptible people. Allergy is an adverse immune reaction to a protein or allergen in our environment that is normally harmless to the non – allergic individual (Kim et al., 2003). Allergy is an irritating or harmful response to a foreign substance that is harmless to most people.

Prurigo is a term frequently incorrectly used to describe chronic itching of any cause. As originally defined by Herba, prurigo denotes papules induced by scratching (Dat et al., 2002). Itch can be defined subjectively as poor localized, non-adopting, usually unpleasant sensation, which elicits a desire to scratch. Mechanisms of itch are both peripheral and central. Impulses of itch pass along the same nerve fiber as responsible for itch of multiracial allergic reaction. Scratching or rubbing seems to give relief by converting the intolerance persistent itch into a more bearable pain and may even cure the itch at the cost of removing the epidermis. No effective specific anti-itch drugs are available.

Purities or itching is a frequent and unpleasant symptom of coetaneous diseases (e.g. Atopic dermatitis, urticaria) and accompanies several systemic disorders (eg. chronic renal failure, cholestasis, diabetes mellitus). Itch associated repetitive scratching often causes skin lesions and

exacerbates the original disease such as atopic dermatitis. Inhibition of itch evoked scratching is consistently beneficial for improving the quality of life of patients and treating the original disease. Inflammation is the stimulation of nerve ending, and is an essential part of body's response to injury or infection. Symptoms of inflammation include redness, swelling, heat and pain. When body tissues are damaged mast cells release a chemical called histamine. Histamine increases blood flow to damaged tissue resulting in redness and heat. Capillaries start leaking blood which result in swelling (Ennis *et al.*, 1980).

## Materials and Methods

### Animals (For anti-allergic activity)

#### Sheep serum method (Noguchi *et al.*, 1990)

Albino rats of either sex weighing between 150 - 250 g were sensitized by injecting subcutaneously 0.5 ml sheep (*Capra hircus*) serum along with 0.5 ml of triple antigen containing toxoids of Diphtheria, Tetanus and Bordetella pertussis organisms 20,000 million.

#### Compound 48/80 induced mast cell degranulation (Lee *et al.*, 1996)

Healthy adult albino rats of either sex of Wistar strain weighing between 150-200 g were selected for the study. Test extracts were given orally to overnight fasted animals.

#### Animals (For anti-pruritic activity) (Ishiguro *et al.*, 2002)

The study was performed on male Wistar albino rats (90 – 120 days old) weighing 150 – 200 g. They were maintained on standard conditions viz., (controlled, humidity and temperature), diet (Hindustan Lever Ltd) and water *ad libitum*. The study was conducted after obtaining institutional animal ethical committee (ref;id-NCP-2006) clearance certificate.

#### Animals (For anti-inflammatory activity) (Ghosh, 1984)

Male albino rats, weighing between 100-150g were used for the experiment.

Experimental procedures were carried out in strict compliance with the Institutional Animal Ethics Committee regulations. The experiment was performed in the morning according to the guidelines for the care of laboratory animals.

**Chemicals:** Compound 48/80 and carageenan were purchased from Sigma Aldrich, USA. Chlorpheniramine maleate, ketotifen fumarate and Ibuprofen were obtained from Pfizer India Ltd. Mumbai. All other reagents used were analytical grade.

**Plant material:** *Centella asiatica* plant (voucher No. ncp/6/2006) was collected freshly from in and around Calicut district of Kerala, India. Plant dried under shade, made into coarse powder by grinding. Plant was identified and authenticated at the herbarium Tamilnadu Agricultural University, Coimbatore.

#### Preparation of plant extract

**Aqueous Extract:** To 20 g of each dried plant powder form, 500 ml water were added and contents of flask were mixed thoroughly by gentle shaking. Flasks were kept for four days with frequent shaking. After the completion of maceration process the filtrate was obtained and water evaporated to get the dried extract. (evaporation by keeping flasks in electric mantle at 80 °C).

The residual extract was dissolved in water and used in the studies.

**Alcoholic (ethanol) extract:** To 20 g of each dried plant powder form, 500 ml ethanol were added and contents of flask were mixed thoroughly by gentle shaking. Flasks were kept for four days with frequent shaking. After the completion of maceration process the filtrates were obtained and solvent evaporated to get the dried extract. (evaporation by keeping flasks in electric mantle at 80 °C).

## Groups and treatment

### Antiallergic activity

#### Sheep serum method

The sensitized rats were divided into four groups of six animals

# Group I : Control, received only vehicle (2% gum acacia solution, 2 ml / Kg p.o)

# Group II : Treated with ketotifen fumarate (1 mg/Kg p.o)

# Group III and # Group IV: Treated with aqueous / ethanol extract of *C. asiatica* (100 and 100mg/Kg p.o).

2. Treatment was continued for 14 days. During the course of treatment the animals were maintained under controlled condition of temperature and were fed with standard diet.
3. On day 14<sup>th</sup>, 2 hrs after the assigned treatment, the rats were sacrificed and the intestinal mesentery was taken for studies on mast cells.
4. The mesenteries of sacrificed rats along with pieces of intestine were kept in Ringer-Locke solution at 37<sup>o</sup> C (NaCl- 9.0 g, KCl- 0.42 g, NaHCO<sub>3</sub>- 0.15 g, glucose 1.0 g, CaCl<sub>2</sub> – 0.250 g/ litre of distilled water) at 37<sup>o</sup> C.
5. The mesenteric pieces were challenged with 5% sheep serum for 10 mins and then transferred to a wide mouthed bottle containing 10% formalin for 24 hrs.
6. The mesenteric fans were fixed dried and stained with toluidine blue (0.1%) on a clean slide. The excess of stain was washed with distilled water followed by dehydration in absolute alcohol. Finally the slides were cleared in xylene and mounted in diphenyl phthalein-xylene and examined microscopically for the number of intact and degranulated mast cells in at least 10 randomly selected high power fields.

#### **Compound 48/80 induced mast cell degranulation**

The animals were divided randomly into four groups and were given different doses of the extracts of *C.asiatica* by oral route.

# Group I: Control, received only vehicle (2% gum acacia solution, 2 ml / Kg p.o)

# Group II : Treated with ketotifen fumarate (1 mg/Kg p.o)

# Group III and # Group IV: Treated with aqueous/ ethanol extract of *C.asiatica* (100 and 100mg/Kg p.o). 10 ml of normal saline was injected into the peritoneal cavity of normal male rats (150-200g) after a gentle massage. The peritoneal fluid was collected and transferred into the siliconised test tubes containing 7-10 ml RPMI-1640 medium (pH 7.2-7.4). Mast cells were washed thrice by centrifugation at low speed (400-500rpm) followed by discarding the supernatant and taking the pellets of mast cells into the medium. These cells were purified and incubated with compound 48/80 (p-methoxy-N-methylphenylamine) (5µg/ml) at 37°C for 10 mins. After the incubation, these cells were spinned and stained with 0.1% toluidine blue solution and observed under a microscope. Test compounds were given to rats orally prior to collection of mast cells in doses of 100mg/kgs for 14 days in two sets of experiments. Control animals received an equal volume of 2% w/v gum acacia solution 2 ml.

#### **Antipruritic activity**

Albino rats of either sex weighing between 120-150 g were divided into four groups.

Group I : Control, received only vehicle (2% gum acacia solution, 2 ml / Kg p.o)

Group II : Treated with Chlorpheniramine maleate (1 mg/Kg p.o)

Group III and Group IV: Treated with aqueous/ethanolic extract of *C. asiatica* (100 and 100mg/Kg p.o).

#### **Assay of antipruritic activity**

The antipruritic activity was evaluated by examining the incidence of scratching. Scratching behaviour was induced by subcutaneous injection of 0.1% solution of compound 48/80 in saline at 100 µl/site into the base of the neck on the back side of the rat. Scratching on the injected site by the hind paws were counted for 30 mins disregarding those at other site such as ears. Test compounds such as ethanol extract and water extract of *C.asiatica* (100 mg/kg and 100 mg/Kg) were given orally 1 hr before the injection of the compound 48/80. As a control, rats were administered 2% gum acacia (2 ml) orally. Chlorpheniramine maleate was used as the reference standard.

#### **Anti-inflammatory activity**

##### **Induction of experimental inflammation:**

All groups were fasted overnight and provided only water. First group served as control which received only 1% sodium carboxy methyl cellulose suspension. Second group served as standard which received ibuprofen at a dose of 100mg/kg body weight/oral. Remaining groups received extracts at a dose of 200mg/kg body weight/oral. One hr after treatment, 0.05ml 1% carageenan suspension was injected subcutaneously in to the sub plantar tissue of the right hind foot. The volume of injected paw was measured after 3hrs. The average paw volume in a group of test compounds treated rats was compared with that of control groups and the percentage inhibition of oedema was calculated using the following formula .

$$\% \text{ inhibition} = 100 (1 - V_t/V_c)$$

$V_t$  = mean volume of paw treated with test compounds

$V_c$  = mean volume of paw treated with control

Male albino rats, weighing between 100-150g were used for the experiment. They were divided into 4 groups of six animals each. All groups were fasted overnight and provided only water. First group served as control which received only 1% sodium carboxy methyl cellulose suspension. Second group served as standard which received ibuprofen at a dose of 100mg/kg body weight/oral. Remaining group received CA Et.

## Results

The results are presented in Tables 1 - 4.

**Table 1:** Effects of extracts of the leaves and flowers parts of CA Et on sheep serum induced mast cell degranulation.

Group	Treatment	Dose	No.of Animals	Intact Cells(%)	Disrupted
1	Control	2% solution	6	22.16±1.29	77.91±0.49
2	Standard(ketotifen)	1 mg/kg	6	75.33±2.99*	24.60±3.37
3	Aqueous extract	100mg/kg	6	54.62±1.08*	39.76±2.14
4	Alcoholic extract	100mg/kg	6	75.31±2.32*	21.62±2.82

\* $p < 0.05$  vs control (ANOVA post hoc Scheffe's test).

**Table 2:** Effect of various extracts of leaves and flowers parts of CA Et on compound 48/80 induced mast cell degranulation.

Group	Treatment	Dose	No.of Animals	Intact Cells(%)	Disrupted
1	Control	2% solution	6	22.00±2.04	77.91±0.49
2	Standard	1 mg/kg	6	69.50±2.85*	30.42±1.84
3	Aqueous extract	100mg/kg	6	51.48±2.72*	44.12±2.14
4	Alcoholic extract	100mg/kg	6	74.5±1.89*	23.75±2.69

•  $p < 0.05$  vs control (ANOVA post hoc Scheffe's test).

**Table 3:** Antipruritic activity of CA extract against compound 48/80 induced pruritis

Group	Treatment	Dose	No.of Animals	Incidence of scratching(Mean ±SE)
1	Control	2%acacia	6	75.33±1.65
2	Chlorpheniramine maleate	0.325mg/kg	6	35.33±1.23*
3	Aqueous extract	100mg/kg	6	31.24±1.26*
4	Alcoholic extract	100mg/kg	6	36.22±1.16*

\* $p < 0.05$  vs control (ANOVA post hoc Scheffe's test).

**Table 4:** Anti-inflammatory activity of CA Et.

Treatment	Dose(mg/kg)	Mean oedema volume ±S.E(ml)After 3 hr	% inhibition of Oedema after 3 hr.
Control	-	0.615±0.014	-
Standard(ibuprofen)	100	0.205±0.023	66.66
Aqueous extract	100	0.192±0.024	46.31
Alcoholic extract	100	0.212±0.031	71.18

## Anti-allergic studies

*Centella asiatica* extracts(aqueous 100 mg/kg) and (alcoholic 100mg/kg) showed a better protection of mast cell degranulation induced by sheep serum (76-83 %) than the standard drug

Ketotifen fumarate(75%). *C.asiatica* extracts also exhibited mast cell stabilizing activity when peritoneal mast cells were stimulated to be degranulated by compound 48/80. Alcoholic and aqueous extracts inhibited mast cell degranulation (75-82%) whereas the reference drug Ketotifen fumarate (1mg/kg) showed 69% protection of mast cells.

#### Anti-pruritic studies

Regarding anti-pruritic activity, Table 3 shows the time course of the anti-pruritic effect produced by the CA Et (aqueous and alcoholic 100mg/kg, positive control like Chlorpheniramine maleate). CA Et decreased the scratching incidence. Subcutaneous administration of the extract resulted in significant decreased in number of the response. Thus CA Et aqueous and alcoholic 100 mg / kg were found to have inhibitory effects on compound 48/80 induced antipruritic activity. Subcutaneous injection of compound 48/80 at a dose of 50 µg/mouse elicited a significant scratching response in rats. CA Et at 100 mg and chlorpheniramine maleate at 0.325 mg/kg significantly inhibited the scratching response.

#### Anti-inflammatory studies

Considering anti-inflammatory activity, Table 4 shows that CA Et possessed anti-inflammatory activity. CA Et 100 mg/kg showed similar degree of activity to the standard Ibuprofen.

## Discussion

The antiallergic, anti-inflammatory and antipruritic properties of the aqueous and alcoholic extracts of the plant taken up in our studies were evaluated using different experimental models. Analysis of results obtained revealed that significant antiallergic activities has been obtained with all the extracts comparable with that of standard drug ketotifen viz., 75% for sheep serum model and 69% for the compound 48/80 model. Glycosides which are normally present in the polar fraction have been reported to possess antiallergic potential.

A variety of *in vitro* and *in vivo* experiments have shown that selected glycosides possess antiallergic, anti-inflammatory, antioxidant, antiviral activities (Gokhale and Saraf, 2000; Gupta and Tripathi, 1993; Mastuda et al., 1997). Certain glycosides possess potent inhibitory activities against a wide array of enzymes such as protein kinase C, protein tyrosine kinase, phospholipase A2 (Shinde et al., 1999; Lewis, 1983). Other glycosides potentially inhibit prostaglandins, a group of pro-inflammatory signaling molecules. This is mainly due to inhibition of key enzymes involved in prostaglandin biosynthesis (lipoxygenase, phospholipase and cyclooxygenase) (Nakahata et al., 2002). Inhibition of this key enzymes provides the mechanism by which glycosides inhibits the inflammatory process.

Analgesic and anti-inflammatory effects have already been associated with glycosides as well as with terpenes (Yadava and Kumar, 1999; Viana et al., 2003). Luteolin has been reported to possess significant antiallergic and anti-inflammatory effect (Inoue et al., 2002).

Stimulation of mast cells with compound 48/80 or antiserum initiate the activation of signal transduction pathway which leads to histamine release. Some recent studies showed that compound 40/80 and other polybasic compounds are able to activate G proteins (Mousli et al., 1990). Anti-allergic, anti-pruritic as well as anti-inflammatory activities of *Centella asiatica* can be attributed to the presence of glycosides like asiaticoside, madecassoside as well as triterpenes.

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