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Anti-inflammatory and anticancer activity of *Heracleum rigens* Wall. ex DC.

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Abstract

Heracleum rigens is found only in few hilly mountains of Western Ghats of India. The genus *Heracleum* is reported to contain many phytoconstituents of medicinal value. Coumarins are one amongst them, which are known to possess anti-inflammatory and cytotoxic properties. The seed and root extracts of *H. rigens* were evaluated for its anti-inflammatory and cytotoxic activities. The anti-inflammatory activity of methanolic extracts of seed and root was evaluated by carrageenan induced paw edema model in Albino Wistar rats. The extracts were also tested for short term cytotoxic property against Dalton's lymphoma ascites cells by trypan blue method. The seed and root extracts showed a significant anti-inflammatory activity. The edema inhibition of seed extract at 200 mg kg^{-1} and 400 mg kg^{-1} were 26.8% and 38.2% respectively after 4h, while the root extract at 200 mg kg^{-1} and 400 mg kg^{-1} were 36% and 39.4% respectively after 4h. The seed and root extracts showed significantly higher levels of cytotoxicity. EC_{50} values of seed and root extracts were 6.9 μg ml^{-1} and 10.8 μg ml^{-1} respectively. *Heracleum rigens* being rich in coumarins is found to be a good source for anti-inflammatory and anticancer agents.

Keywords: *Heracleum rigens;* anti-inflammatory, anticancer

Introduction

Plants have been used for the treatment of many medical disorders for centuries and have been the source of several clinically useful cytotoxic and anti-inflammatory agents. Majority of the plants belonging to the family Apiaceae are aromatic with hollow stems commonly known as umbellifers, used as traditional ethno medical remedies and more than 100 cultivated species are registered for several uses (Ana et al., 2010). *Heracleum rigens* is

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a perennial herb belonging to Apiaceae disturbed in the high altitudes of Western Ghats of India. In Ayurveda, *Heracleum rigens* has been traditionally used for urinary disorders, cough, hyperacidity, wounds, pruitus abdominal disorders, and cardiac diseases and vomiting. In Siddha, it is used for treating constipation, stomachache, diarrhea, headache, phlegm, gastric disorders and indigestion (Yoganarasimhan., 1996). *Heracleum rigens* is reported to contain a group of phyto-constituent called as coumarins (Saraswathy et al., 1990). Coumarins comprise a very large class of phenolic derivatives and consist of fused benzene and α-pyrone rings. To date, more than 1300 types of coumarins have been identified, chiefly as secondary metabolites in green plants, fungi and bacteria, possessing anti-inflammatory and cancer chemopreventive properties (Iranshahi et al., 2009). Species such as *H. persicum* is reported to have anti-inflammatory activity (Valiollah et al., 2009), while 5-(3-methyl but-2-enyloxy) 7 methoxy coumarin, isopimpinellin and 8-hydroxy, 5-methoxy furanocoumarin were reported in *Heracleum rigens* (Saraswathy et al., 1990). Despite its extensive use in traditional treatment, detailed studies focusing on anti-inflammatory and anticancer properties have not been carried out. In view of this, in the present study an attempt is made to evaluate the anti-inflammatory and cytotoxic activities of methanolic extracts of seed and root of *Heracleum rigens.*

Materials & Methods

Chemicals

Carrageenan, Diclofenac sodium and Trypan blue were purchased from Sigma-Aldrich Co. India. All other reagents and chemicals used were of analytical grade.

Plant material

Heracleum rigens fresh plant material collected from the Bababudangiri hills of Western Ghats, were identified using Flora of Coorg (Keshava et al., 1990). Further authentication was done at National Ayurveda Dietetics Research Institute (Central Council for Research in Ayurveda and Sidda) with reference No-Drug authentication/SMPU/NADRI/B-GN/2010-11/491 and the voucher specimen is deposited in the department herbaria.

Preparation of plant extracts

Methanolic seed (MSE) and root extracts (MRE) were prepared by soaking 200 g of air dried powdered material in 750 ml of absolute methanol. The dynamic maceration was carried out for 7 days at room temperature and same procedure was repeated thrice until extraction was complete. The extracts were filtered and concentrated under reduced pressure in vacuum rotary evaporator. The resulting extracts were stored in 4˚C. The extracts were screened for the presence of coumarins by treating the alcoholic solution of extracts with alkali and observed for the florescence.

Animals

Albino Wistar rats of either sex and approximately of same age (12 to13 weeks), weighing between 150-200 g were used for the animal studies. They were maintained under

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controlled conditions of temperature (23±2˚C) and humidity (55±5%). They were fed with commercially pelleted rat feed with water *ad libitum.* The anti-inflammatory activity was conducted after obtaining the approval from Institutional Animal Ethical Committee (IAEC/-05/KLEB/2011). CPCSEA guidelines were followed during the maintenance and experiment.

Acute toxicity studies

Acute oral toxicity study of methanolic extracts of *Heracleum rigens* was carried out according to OECD guidelines 423 (OECD 2000). Observations were recorded systematically.

Carrageenan induced paw edema

Albino Wistar rats weighing between 150-200 g were randomly divided into six groups of six animals each. Group I served as control, Group II to Group VI were the treated sets and received seed extract (200 mg kg^{-1} and 400 mg kg^{-1}), root extract (200 mg kg^{-1} and 400 mg kg^{-1}) and diclofenac sodium (15 mg kg^{-1}) respectively. After 1 h of drug administration 0.1ml of 1% suspension of carrageenan was injected into the sub-plantar region of the right hind paw of the each rat. The paw volumes were measured using a plethysmograph immediately after injection and every 1 hour up to 5 hours (Winter et al., 1962). The percentage inhibition in paw volume was determined using the formula: (Control reading -Test reading) /Control reading×100.

Anticancer activity

The methanolic seed and root extracts of *H. rigens* were evaluated for short term *in-vitro* cytotoxicity using Dalton's lymphoma ascites cells. The cell viability was checked by trypan blue dye (1%). The cell suspension (1x 10^6cells in 0.1 ml) was added to tubes containing various concentrations of the test compounds ranging from 10, 20, 50, 100 and 200µg. The volume was made up to 1ml using phosphate buffered saline (PBS). Control tube contained only cell suspension. These assay mixtures were incubated for 3 h at 37°C. After incubation, 0.1 ml trypan blue was added and number of dead cells was determined using haemocytometer by trypan blue exclusion method (Kuttan et al., 1985).

Statistical analysis

Statistical analysis was performed using One-way ANOVA followed by Dunnett's test to determine the significance of difference among the various treated groups and the control group.

Results

Screening of coumarins

Preliminary screening showed the presence of coumarins in the seed and root extracts of *H. rigens.*

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Acute toxicity studies

Animals were treated with extracts up to a maximum dose of 2000 mg/kg. There was no mortality or any behavioral changes amongst the animals tested. The acute toxicity test indicated that LD_{50} of seed and root extract of *H. rigens* were found to be safe up to 2000 mg/kg. The anti-inflammatory evaluation was carried out at 200 mg kg^{-1} and 400 mg kg^{-1} using carrageenan induced paw edema model.

Anti-inflammatory studies

In carrageenan induced paw edema in rats, oral administration of methanolic extracts of seed and root showed inhibition of paw edema at 2, 3, 4 and 5 h after carrageenan injection and was compared with standard diclofenac sodium (Table 1). Orally administered doses 200 mg/kg and 400 mg/kg of seed extract showed significant inhibition ($p<0.01$) dose dependently. While the methanolic root extract of doses 200 mg $kg^{-1}$and 400 mg kg^{-1}showed significant inhibition ($p<0.01$), however higher degree of dose dependency was not recorded. The anti-inflammatory effect induced by diclofenac sodium progressively increased and reached a maximum of 51.4% after 4hrs ($p<0.001$). Both the extracts at 400 mg kg^{-1}showed better inhibition of edema than dose at 200 mg kg^{-1}. Sub-plantar injection of carrageenan in rats showed a time dependent increase in paw thickness which was observed at 1 hour and was maximal at 3 hour in the control group.

Anticancer activity

The seed and root extracts of *H. rigens* showed a potential cytotoxic activity against Dalton's lymphoma ascites cells. The EC_{50} of methanolic seed extract (MSE) and root extract

Table 1. Effect of methanolic extracts of *H. rigens* on Carrageenan induced paw edema in rats.

Treatment	Rat hind paw volume in ml (percentage inhibition)				
	1hr	2hr	3hr	4hr	5hr
Control	0.650 ±0.022	0.820 ±0.031	0.930 ±0.033	0.875 ±0.033	0.780 ±0.031
Diclofenac sodium (15 mg kg^{-1})	0.55 ±0.022*** (15%)	0.600 ±0.001*** (26.8%)	0.475 ±0.017*** (48.9%)	0.425 ±0.017*** (51.4%)	0.400 ±0.022*** (48.6%)
MSE (200 mg kg^{-1})	0.620 ±0.028* (4.6%)	0.670 ±0.021* (15.8%)	0.770 ±0.038* (17.2%)	0.640 ±0.022* (26.8%)	0.520 ±0.022* (33%)
MSE (400 mg kg^{-1})	0.570 ±0.021** (12.3%)	0.630 ±0.036** (23.1%)	0.590 ±0.037** (36.5%)	0.540 ±0.030** (38.2%)	0.460 ±0.020** (41%)
MRE (200 mg kg^{-1})	0.640 ±0.035* (1.5%)	0.680 ±0.017* (17%)	0.640 ±0.035* (32.2%)	0.560 ±0.037* (36%)	0.490 ±0.020* (37.1%)
MRE (400 mg kg^{-1})	0.580 ±0.017** (10.7%)	0.600 ±0.027** (26.8%)	0.620 ±0.025** (33.3%)	0.530 ±0.025** (39.4%)	0.470 ±0.025** (39.7%)

Values are mean ± SEM, n=6. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, One-way ANOVA followed by Dunnett's test; MSE : Methanolic Seed Extract ; MRE: Methanolic Root Extract.

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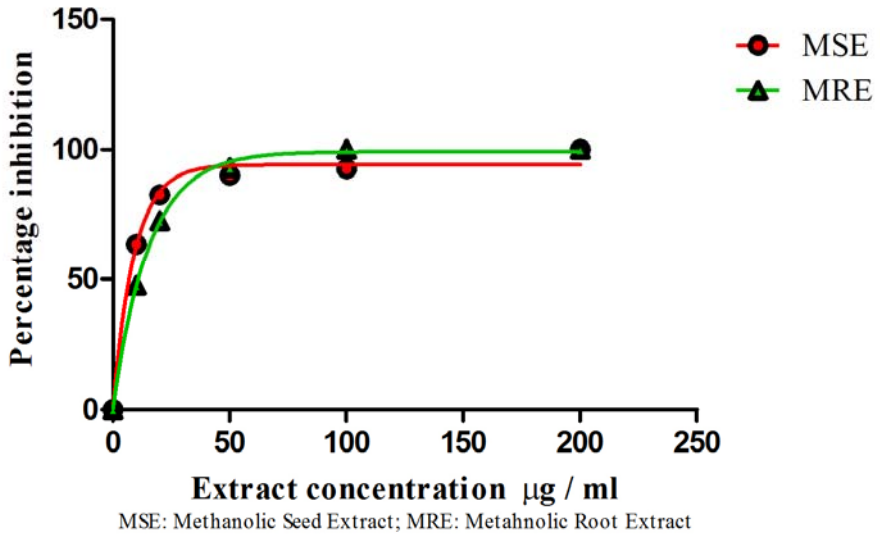

MSE: Methanolic Seed Extract; MRE: Metahnolic Root Extract

Figure 1. Effect of methanolic extract of *H. rigens* on the Dalton's lymphoma ascites cells.

(MRE) were found to be 6.9µg ml^{-1} and 10.8 µg ml^{-1} respectively. There was a graphical increase in the percentage of cytotoxicity with increasing concentration of the extracts (Figure 1). The seed and root extracts showed 100% cytotoxicity at 200 µg ml^{-1} after 3 hours of incubation. The lower EC_{50} represents the higher potency of the extract to inhibit the growth of cells and cause toxicity. The seed extract showed lower EC_{50} than the root extract.

Discussion

Edema formation in rats is biphasic event, early phase results in the production of histamine, serotonin and possibly the cylooxygenase products and kinins like substances at the peak of 3rd hour. The second phase of edema is due the release of prostaglandins, free radicals, proteases and lysosomes. The second phase is sensitive to most of the clinical anti-inflammatory drugs (Perianayagam et al., 2006; Vinegar et al., 1969).

Non-steroidal anti-inflammatory drugs (NSAID) block the synthesis of prostaglandins by inhibiting cyclooxygenase (COX). COX and 5-lipoxygenase (5-LO) catalyzes the peroxidation of arachidonic acid. Polyphenols like coumarins and flavonoids might be expected to interfere with this process (Hoult et al., 1994). Coumarin and umbelliferone were reported to have a mechanism of action similar to NSAID in a carrageenan induced inflammation and the effect lasted for at least 3 h, which is the time for the maximum effect of carrageenan (Lino et al., 1997). Carrageenan-induced rat paw edema has been inhibited also by ethanol root extract of *Peucedanum ostruthium* where 6-(3-carboxybut-2-enyl)-7-hydroxycoumarin is implicated as the anti-inflammatory compound (Hiermann et al., 1998). Compounds such as columbianadin, columbianetin acetate, bergapten and umbelliferone isolated from *Angelica pubescens* demonstrated both anti-inflammatory and analgesic activities at 10 mg kg^{-1} in mice (Chen et al., 1995). Marmim, a coumarin isolated from the roots of *Aegle marmelos* contributed to the anti-inflammatory activity (Shoeb et al., 1973). The furanocoumarin sphondin from *H. laciniatum* is reported to have inhibitory effect on IL-1β-induced COX-2 protein and PGE_2 (Ling-LY et al., 2002). The methanolic seed extract of *H. rigens* inhibited the edema formation in Albino Wistar rats significantly in the 2nd phase. A study conducted by Valiollah et al., 2009 using methanolic seed extract of *H. persicum* showed edema inhibi-

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tion percentage of 23% and 43% at 200 mg $kg^{-1}$and 400 mg kg^{-1}respectively. The powdered coriander fruits exhibited anti-inflammatory effects on carrageenan induced oedema in rat paw at both 200 and 500 mg kg^{-1}doses (Ammar et al., 1997).

The methanolic root extract of *H. rigens* similarly showed inhibition of paw edema formation in the 2nd phase. The aqueous root extract of *A. marmelos* also showed significant inhibition of carregenan induced paw edema at 100 mg kg^{-1} (Jyoti et al., 2011). The present study reveals that *H. rigens* extracts does contain compounds which inhibit inflammation mediators and prostaglandin synthesis pathway, thus inhibiting the inflammation formation. This inhibition effect could be due the presence of coumarin compounds in the extracts of *H. rigens.*

The present study also revealed that seed and root extracts possess significant cytotoxic property since both the extracts contain coumarins. *H. rigens* showed better cytotoxic activity against Dalton's lymphoma ascites cells when compared with other Apiaceae members namely *Centella asiatica, Coriandrum sativum, Cuminum siminium* and *Foeniculum vulgare* (Babu et al., 1995). Ethanolic fruit extract of *H. sibirium* showed much higher EC_{50} values against the different human leukaemia cell lines (Bogucka-Kocka et al., 2008).

The criterion for cytotoxic and non-cytotoxic was adapted from the guidelines set by the National Cancer Institute (NCI), United States of America. The screening protocol according NCI has indicated that plant or animal extracts with $EC_{50} \leq 20\mu g\ ml^{-1}$ were considered to be cytotoxic and non-cytotoxic if otherwise (Geran et al., 1972). The seed and root extracts of *H. rigens* produced 50% cell death at very low concentrations. The EC_{50} value of the seed and root extracts were below 20 $\mu g\ ml^{-1}$. This implies that *H. rigens* extracts are highly cytotoxic.

The present study indicates that the methanolic extracts of the seed and root of *H. rigens* possess significant anti-inflammatory and cytotoxic activities. Although further investigation is required to find out the specific active compounds, it is suggested that *H. rigens* is a good source of anti-inflammatory and cytotoxic agents.

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Conflict of Interest statement

There is no conflict of interest associated with the authors of this paper.

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