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Analgesic and Anti-inflammatory Activity of *Carissa carandas* Linn fruits and *Microstylis wallichii* Lindl Tubers

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Abstract – The ethanolic (50% v/v) extracts of *Carissa carandas* (fruits) (Apocynaceae) and *Microstylis wallichii* (tubers) (Orchidaceae) were examined for anti-inflammatory and analgesic activities in experimental animals. *Carissa carandas* and *Microstylis wallichii* (50 - 200 mg/kg) caused a dose dependent inhibition of swelling caused by carrageenin significantly in cotton pellet induced granuloma in rats ($P < 0.05$ to $P < 0.001$). There was a significant increase in the analgesy meter induced pain in rats. The extracts of *Carissa carandas* and *Microstylis wallichii* resulted in an inhibition of stretching episodes and percentage protection was 16.05 - 17.58% respectively in acetic acid induced writhing.
Keywords – *Carissa carandas, Microstylis wallichii*, pain, inflammation

Introduction

Carissa carandas Linn (Apocynaceae) is an evergreen diffuse and spiny shrub occurring through out the country. The plant is very valuable for the Indian System of medicine particularly *Ayurveda*. It is used for alleviating *vata* and *pitta* disorders. Its fruits and seed latex are used for treating rheumatoid arthritis, anorexia, indigestion, colic, hepatomegaly, splenomegaly, piles, cardiac diseases, oedema, amenorrhoea, fever and nervine disorder (Pushpangadan, 2003). The roots are useful in stomach disorder, intestinal worms, Scabies, diabetic, ulcer and pruitis (Sharma *et al.*, 2001). Alcoholic extract of the root exhibits cardiotonic effect (Vohra and De., 1963) anti-hypertensive properties. *Microstylis wallichii* Lindl (Orchidaceae), which is classified under the *Astavarga* in Ayurveda and used as one of the major constituent in various formulations (Chopra *et al.*, 1956) such as *Chavanprash*, *Bramrha Rasayan* and *Haritak*-Kyadiyoga (Chunekar, 1982). It is a small herb growing in north temperate and subtropical Himalayas from Shimla to Sikkim at an altitude of 11 - 1200 meters. In this study we aimed to provide more information on the fruits of *Carissa carandas* and tubers of *Microstylis wallichii* of the plant by investigation some of its pharmacological effects to provide a scientific validation for the acclaimed medicinal use.

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Experimental

Plant materials – The fruits of *C. carandas* were purchased from the local market, Lucknow U.P. and tubers of *M. wallichii* were supplied by Regional Research Institute, Tarikhet (Ranikhet), Uttranchal. The plant material were identified and authenticated taxonomically by Drug Standardisation Research Unit, C.R.I. (ay), Lucknow. A voucher specimen of the collected sample was deposited in the institutional herbarium for future reference.

Preparation of 50% ethanolic extract – The shade dried plant materials was crushed, powdered and exhaustively extracted by overnight maceration with 10 volumes of 50% ethanol. The extracts were filtered, pooled and concentrated on rotavapour (Buchi, USA) and dried in lyophilizer (Laboconco, USA) under reduced pressure to obtain 10% of solid residue (Rao *et al.*, 2003).

Preliminary phytochemical analysis – Preliminary qualitative phytochemical screening (Trease and Evans, 1983) of fruits of *C. carandas* were positive for alkaloids, carbohydrate, tri-terpenoids, proteins, resins and saponin. *M. wallichii* were positive for alkaloids, carbohydrate resin and saponin.

Test animals

Animals – Sprague-Dawley rats (140 - 160 g) and albino mice (18 - 24) of either sex were purchased from the animal house of National Laboratory Animal Centre, Lucknow, India. All experiments were performed in the morning according to current guidelines for the care of laboratory animals and the ethical guideline for investigation of experimental in conscious animal (Zimmerman, 1983)

Experimental procedure – *C. carandas* and *M. wallichii* (suspended in 0.5% carboxy methyl cellulose in distilled water) was administered once daily in dose of 50 - 200 mg/kg for 3 consecutive days. Phenylbutazone (Sigma, USA) in the dose of 100 mg/kg p.o, was used as the standard anti-inflammatory and analgesic agent. The reference drug was administered 30 minutes before the experiment. Control group of animals received (according to body weight) suspension of 0.5% carboxy methyl cellulose in distilled water. Experiments were conducted on day 3, one hour after last drug or vehicle administration.

Evaluation of anti-inflammatory activity-λ Carrageenin-induced paw oedema – Rats were injected with 0.1 ml of 1% λ carrageenin into the subplantar side of the left hind paw (Winter *et al.*, 1962). The paw was marked with ink at the level of lateral malleolus and dipped in perspex cell up to this mark. The paw volume was measured immediately with an Ugo Basile Plethysmometer (No: 61402, 7140 Comerio-varese, Italy) and 3 h after injecting the λ carrageenin suspension. The difference was recorded as volume of oedema that attains the maximum inflammation in paw with λ-carrageenin. The extracts were administered orally at the doses of 50, 100 and 200 mg/kg respectively by gavage 1 h before the λ carrageenin injection. Significant reductions in the paw volume compared to vehicle treated control animals were considered as anti-inflammatory response. Percentage inhibition of oedema was calculated as follows:

$$\% \text{ Inhibition} = (1 - VT/VC) \times 100$$

VT = Paw volume in drug treated rats.

VC = Paw volume in control group of rats.

Cotton pellet induced granuloma formation – The rats were anesthetized with ether and incision was made on the lumbar region (Winter and Porter, 1957). By a blunted forceps subcutaneous tunnel was made and a sterilized cotton pellets (100 mg ± 1 mg) was inserted in the groin area. The animals were treated for seven days at the doses of 50, 100 and 200 mg/kg body weight by oral route. The animals were sacrificed on the 8th day and, the pellets were removed and dried until the weight remained constant according to the procedure described (Sheth *et al.*, 1972) and the net dry weight was calculated.

Evaluation of antinociceptive activity

Analgesy-meter induced pain – The analgesic effect of the fruits of *C. carandas* and tubers of *M. wallichii* were tested in mice of either sex, using an Ugo Basile Analgesy meter (No. 32725, 21025 Comerio-varese, Italy) (Rodriguezalia, 1990; Rao *et al.*, 2003). This method involves the application of force to the paw of the mice using the analgesy-meter, which exerts a force at a constant rate. The mice were gently placed between the plinth and plunger. The instrument was switched on and a constant motor rate was used to drive the plunger on to the paw of the mice. When the mice struggled, the instrument was switched off and the force at which the animal felt pain was read on a scale calibrated in grams x 10 by a pointer. The pre and the post treatment weight causing pain were determined for each mouse. The extracts of *C. carandas* and *M. wallichii* administration (50, 100 and 200 mg/kg; oral) and morphine sulphate (10 mg/kg) were administered intra peritoneally 30 minutes before testing at doses of 50, 100 and 200 mg/kg respectively.

Acetic acid induced writhing – The analgesic effect of the fruits of *C. carandas* and tubers of *M. wallichii* were studied by the reduction of acetic acid induced writhing in the mice (Witkin *et al.*, 1961). After 30 minutes of *C. carandus* and *M. wallichii* administration (50, 100 and 200 mg/kg; oral) or the reference standard, the animals received 10 ml/kg acetic acid (0.6%, i.p.). The number of abdominal contractions (writhings) and stretching with a jerk of the hind limb were counted for 15 min after administering acetic acid and percent inhibition was calculated.

Gross behavior and acute toxicity studies – Different doses (50 - 2000 mg/kg, p.o.) of *C. carandas* and *M. wallichii* were administered to groups of 10 mice of each dose, while one group of the same number of mice served as control. The animals were observed continuously for 1 h and then at half-hourly intervals for 4 h, for any gross behaviour changes, inducing general motor activity, writhing, convulsions, response to tail pinching, piloerection, pupil size, fecal output and feeding behaviour and further up to 72 h and 15 days for any mortality (Miller and Tainter, 1994).

Statistical analysis – All the data were presented as mean S.E.M. and analysed by Wilcoxon sum rank test (Padmanabhapillai *et al.*, 1982) followed by unpaired students 't' test for the possible significant identification between the various groups. A value of $P < 0.05$ was considered statistically significant.

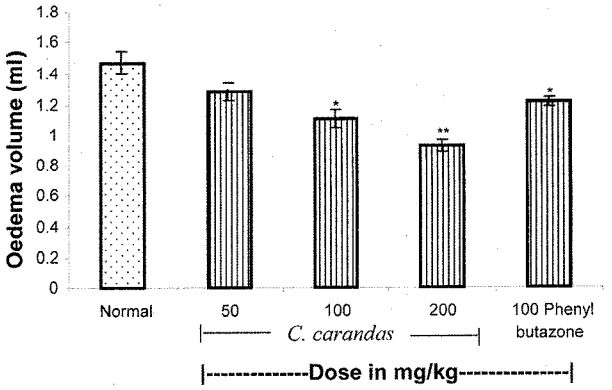

Fig. 1. Effect of *C. carandas* on λ-carrageenin induced paw oedema in rats. Each bar represents mean ± S.E.M. for six rats per group. *P < 0.01, **P < 0.001 compared with the value in normal group.

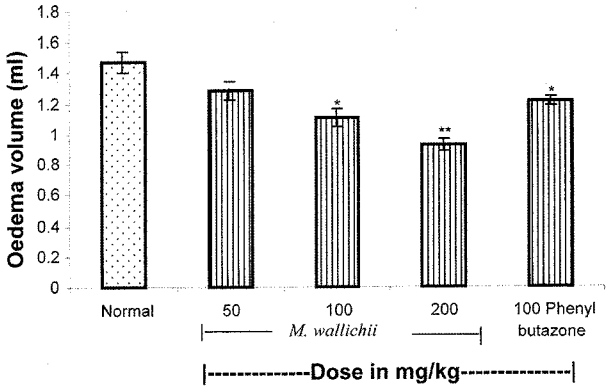

Fig. 2. Effect of *M. wallichii* on λ-carrageenin induced paw oedema in rats. Each bar represents mean ± S.E.M. for six rats per group. *P < 0.01, **P < 0.001 compared with the value in normal group.

Results

Carrageenin induced paw oedema in rats – Oral administration of *C. carandas* and *M. wallichii* dose dependently inhibited the oedema caused by carrageenin in rats. The extract of *C. carandas* and *M. wallichii* at the doses of 50, 100 and 200 mg/kg reduced the oedema and it was 1.28 ± 0.06, 1.10 ± 0.06; 1.29 ± 0.07, 1.12 ± 0.08 and 0.94 ± 0.04; 0.96 ± 0.05 respectively as compared to control 1.48 ± 0.07. Phenyl butazone at the dose of 100 mg/kg significantly decreased the oedema to 1.21 ± 0.03 (Fig. 1, 2).

Cotton pellet induced granuloma in rats – *C. carandas* and *M. wallichii* (50 - 200 mg/kg, p.o.) significantly decreased the granuloma dry weight from 28.5 ± 2.2 –22.4 ± 1.8; 28.7 ± 2.3 – 22.6 ± 1.7 and the results are compared to the reference compound phenyl butazone (Fig. 3, 4).

Acetic acid induced writhing - The *C. carandas* and

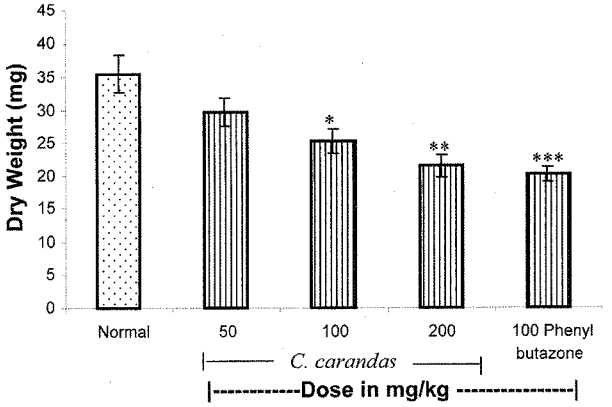

Fig. 3. Effect of *C. carandas* on cotton pellet induced granuloma in rats. Each bar represents mean ± S.E.M. for six rats per group. *P < 0.05, **P < 0.01, ***P < 0.001 compared with the value in normal group.

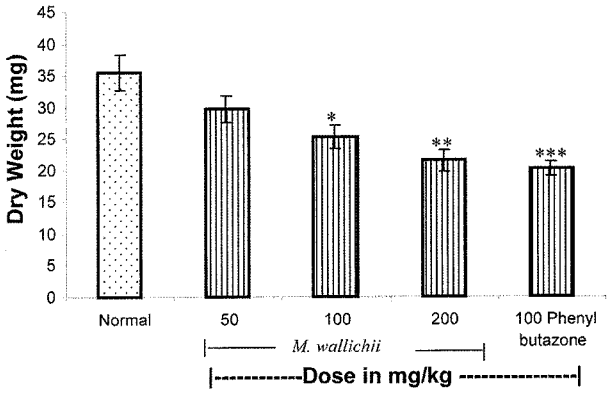

Fig. 4. Effect of *M. wallichii* on cotton pellet induced granuloma in rats. Each bar represents mean ± S.E.M. for six rats per group. *P < 0.05, **P < 0.01, ***P < 0.001 compared with the value in normal group.

M. wallichii extract on writhing response in mice is shown in Fig. 5, 6. It was found that the ethanolic extract caused an inhibition on the writhing reponse induced by acetic acid (0.06%). Increase in the doses of plant extract resulted in the greater inhibition of stretching episodes and the protection ranged 15.51 - 57.84% respectively while phenyl butazone could block the writhing response by 42.39%.

Force induced pain – *C. carandas* and *M. wallichii* in the dose range of 50 to 200 mg/kg, p.o. showed significant increase in the pain threshold from 96.5 ± 4.7 to 132.5 ± 5.4 and protection range 16.05 - 17.58% respectively (Fig. 7, 8).

General gross behavior and acute toxicity studies – *C. carandas* and *M. wallichii* upto 2000 mg/kg body weight, orally showed no gross avoidance of any abnormalities or mortality in mice upto end of the observation period.

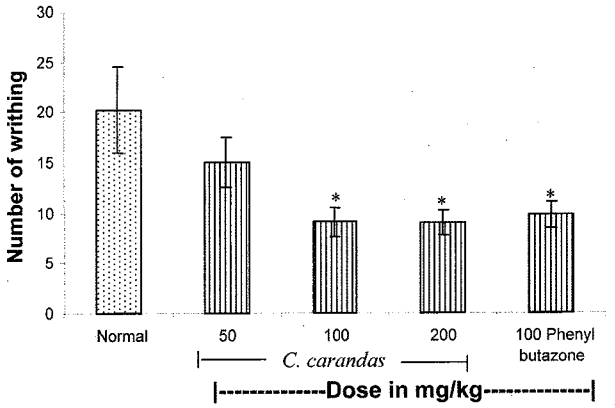

Fig. 5. Effect of *C. carandas* on acetic acid induced writhing in mice. Each bar represents mean ± S.E.M. for six rats per group. *P < 0.05 compared with normal group.

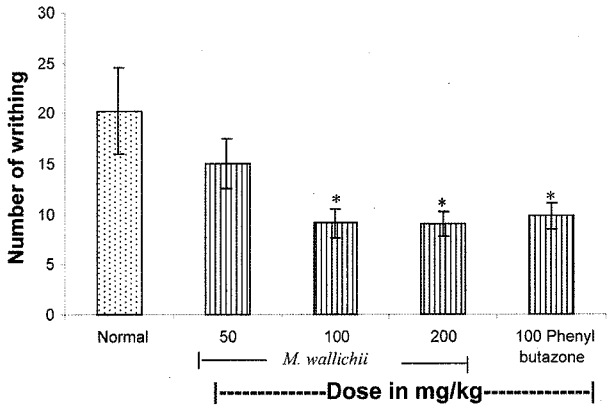

Fig. 6. Effect of *M. wallichii* on acetic acid induced writhing in mice. Each bar represents mean ± S.E.M. for six rats per group. *P < 0.05 compared with normal group.

Discussion

The present study establishes the anti-inflammatory and analgesic activity of 50% ethanolic extracts of fruits of *C. carandas* and tubers of *M. wallichii*. It is evident that λ carrageenin is a sulphated polysaccharide obtained from sea weed (Rhodophyceae) and is commonly used to induce acute inflammation and is believed to be bi-phasic. The first phase is due to release of histamine and serotonin. The second phase is caused by the release of bradykinin, protease, prostaglandin and lysosome (Castro *et al.*, 1968). It has been reported that second phase of oedema is sensitive to most clinically effective anti-inflammatory drugs, which has been frequently used to access the anti-oedematous effect of natural products (Della Loggia *et al.*, 1968; Alcaraz and Jimenez, 1988). Prostaglandins play a major role in the development of second phase of reaction that is measured at 3 h time. These mediators take part in the inflammatory response

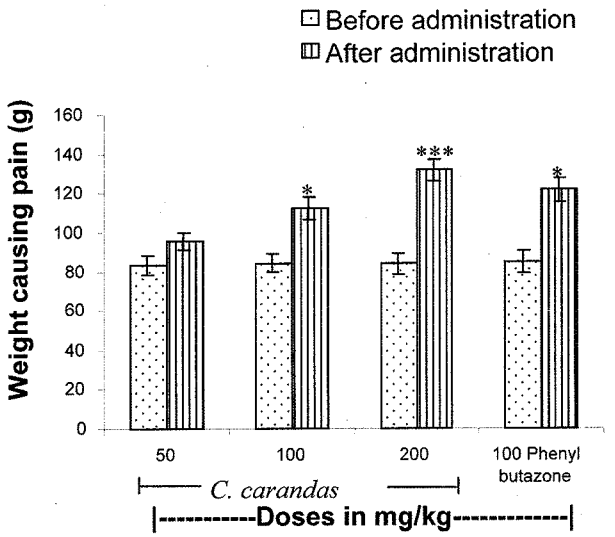

Fig. 7. Effect of *C. carandas* on force induced pain in mice. Each bar represents mean ± S.E.M. for six rats per group. **P < 0.01, ***P < 0.001 compared with the before administration group.

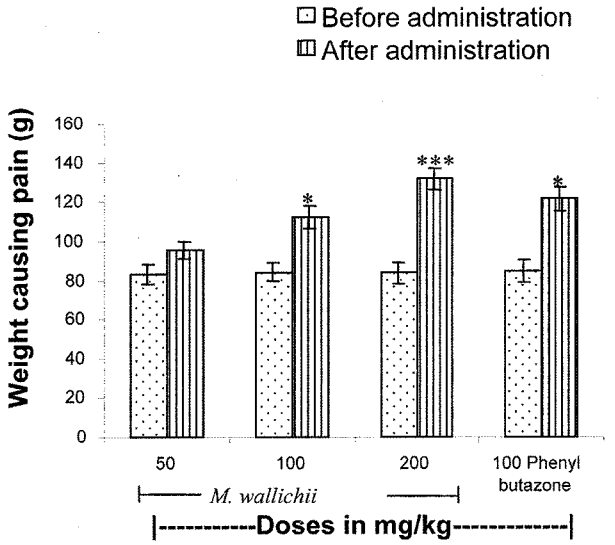

Fig. 8. Effect of *M. wallichii* on force induced pain in mice. Each bar represents mean ± S.E.M. for six rats per group. **P < 0.01, ***P < 0.001 compared with the before administration group.

and are able to stimulate nociceptors and thus induce pain (Di Rosa, 1972). Based on these reports, it can be inferred that the inhibitory effect of *C. carandas* and *M. wallichii* on carrageenin-induced inflammation in rats may be due to inhibition of the mediators responsible for inflammation and pain. Carrageenin induced for edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non steroidal anti-inflammatory agents that is primarily inhibit the enzyme cyclooxygenase in prostaglandin synthesis (Phadke and Anderson, 1988). Based on these reports it can be

inferred that the inhibitory effect of *C. carandas* and *M. wallichii* extracts on carrageenin induced inflammation in rats could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis.

In cotton pellet induced granuloma model of sub-acute inflammation, fruits of *C. carandas* and tubers of *M. wallichii* extracts significantly reduced the weight of granulation tissue. This method was first described by Meier *et al.* (1950), shown that foreign body granulomas were provoked in rats by subcutaneous implantation of pellets of compressed cotton. This method has been useful for evaluation of steroidal and non-steriodal anti-inflammatory drugs (Vogel and Vogel, 1998). Acetic acid causes an increase in peritoneal fluids of PGE2 and PGF2α involving in part, peritoneal receptors (Deraedt *et al.*, 1980; Bentley *et al.*, 1983), and is very sensitive method of screening anti-nociceptive effect of compounds (Collier *et al.*, 1968). The ability of the extracts of fruits of *C. carandas* and tubers of *M. wallichii* in analgesic activity may be due to the involvement of endogenous prostaglandins. However, *C. carandas* and *M. wallichii* alleviated the pain threshold on analgesy-meter induced pain. This offers new perspective in treatment of pain, as there is evidence that symptoms of vital pain varies in intensity of the pain threshold.

Based on the results of the present study it can be concluded that *C. carandas* and *M. wallichii* has potential anti-inflammatory activity against both exudative-proliferative and sub-chronic phases of inflammation. The extracts also have analgesic activity, which is both centrally and peripherally mediated. Thus, using the very small doses given orally in the different validated experimental models; is encouraging enough to warrant further studies and to explore its possible therapeutic role as an anti-inflammatory and analgesic activity in modern clinical practice.

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