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Anti-inflammatory and Antinociceptive Activities of "*Coccinia indica* W. &A." Fruit Juice Powder in Animals

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Abstract – The fresh fruit juice powder of *Coccinia indica* W.&A., (Cucurbitaceae, CJP) was studied for the possible activities of antiinflammatory and antinociceptive to rationalize the folkloric use of the plant juice as rasayana. CJP at the doses of 50-200 mg/kg caused a significant ($P<0.05$ to $P<0.001$) inhibition of paw edema induced by λ carrageenin (1%) and histamine (10^{-3} g/ml, 0.1 ml) in rats. The effect was comparable to the standard cycloxygenase inhibitor brufen at 100 mg/kg and protective percentages were 63.41% and 65.78% respectively. Administration of CJP (50-200 mg/kg) exhibited a moderate increase of the pain threshold on analgesy-meter induced mechanical pain. However CJP significantly prevented the writhing induced by acetic acid in mice and the percentages of inhibitions were 16.98%-35.47%, which is equivalent to 36.67% produced by brufen. These data indicate that the fruit juice of *Coccinia indica* rationalizes the traditional system of medicine.
Keywords – *Coccinia indica*, juice, pain, inflammation.

Introduction

Non-steroidal antiinflammatory drugs (NSAIDs) constitute one of the major groups of drugs that are often used for the treatment of inflammation and pain. However, it is known that most NSAIDs exert potentially adverse effect on the gastrointestinal tract, mainly manifested as gastric ulcers. In the recent years attempts have been made to mask ulcerogenic effect of NSAIDs without affecting their pharmacological actions. *Coccinia indica* W. & A (cucurbitaceae) is a perennial creeping herb with long tapering tuberous roots and grows abundantly in India. The plant occurs both in bitter and non-bitter forms. The green immature fruits of the bitter variety are extremely bitter in taste but they loss their bitterness rapidly during ripening and ultimately become sweet to taste and scarlet in colour. These fruits are reported (Nadkarni 1954, Kirtikar *et al.*, 1933) to be useful in various ailments. The plant is known to possess good hypoglycemic properties (Pillai *et al.*, 1980, Shaw *et al.*, 1981). In this paper we report the anti-inflammatory and antinoceptive activities of the fresh unripe sweet variety fruit juice powder of the *Coccinia indica.* As there is no reference in literature regarding the anti-inflammatory aspects, it was, therefore, considered worthwhile to study the antiinflammatory, analgesic activity and acute toxicity of *Coccinia indica* fruit juice powder in experimental animals.

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Materials and Methods

Plant material – *Coccinia indica* W.&A was collected from the botanical garden of National Botanical Research Institute, Lucknow, India (NBRI) in August 2002. The plant material was identified and authenticated taxonomically at NBRI. A voucher specimen of the collected sample was deposited in the institutional herbarium for future reference.

Preparation of fruit juice powder – The fresh fruits of *Coccinia indica* (1 Kg) were washed with distilled water to remove dirt and soil, size reduced (3-5 cm), crushed and soaked in distilled water (1 L) for 24 h and extracted by a juicer and filtered through muslin cloth. One Kg of fruits yielded 850 ml of juice. The juice was passed through the SD-05 spray drier (Lab-Plant Ltd. U.K.) and the solid content in terms of powder weight obtained was 12% (w/w). The fruit juice powder of *Coccinia indica* (CJP) was stored in a desiccator and used for further experiment after suspending in aqueous carboxymethyl cellulose (1% w/v, CMC).

Test animals – Sprague-Dawley rats (120-150 g) and albino mice (15-20 g) of either sex were purchased from

the animal house of the Central Drug Research Institute, Lucknow. They were kept in departmental animal house in well cross-ventilated room at 26±2°C, and relative humidity 44-56%, light and dark cycles of 10 and 14 h respectively for one week before and during the experiments. Animals were provided with standard rodent pellet diet (amrut, India) and the food was with drawn 18-24 h before the experiment thought, water was allowed ad-libitum.

Antiinflammatory effect

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

$$\% \text{ Inhibition} = (1 - V_T/V_C) \times 100$$

V_T = Paw volume in drug treated rats.

V_C = Paw volume in control group of rats.

Histamine-induced paw edema in rats – The antiinflammatory effect of the CJP against histamine induced paw edema was studied in rats by the method of Parmar and Ghosh (Parmar and Ghosh, 1998). The animals were treated at dose of 50, 100 and 200 mg/kg as described in the previous experiment, except that histamine (10^{-3} g/ml, 0.1 ml) was used as inflammatory agent. The paw volume compared to vehicle treated control animals were considered as antiinflammatory response. Percentage inhibition was calculated as described earlier.

Antinociceptive effect

Analgesy-meter induced pain in mice – The analgesic effect of the powder was tested in mice of either sex, using an Ugo Basile Analgesy meter (No. 32725) (21025 Comerio-varese, Italy) (Spadaro, 1987). This method involves the application of force to the paw of the mice using the Analgesy-meter, which exerts a force that increases 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 struggles, the instrument is switched off and the force at which the animal felt pain was read on a scale calibrated in grams X 10 by a pointers. The pretreatment and the after treatment weight causing pain was determined for each mice. The powder obtained from juice of *Coccinia indica* was administered at a dose of 50, 100 and 200 mg/kg respectively 30 min before testing.

Acetic acid induced writhing in mice – Analgesic activity of CJP was studied by reduction of the acetic acid induced writhing in the mice (Witkin *et al.*). Thirty minutes after the administration of the CJP (50, 100 and 200 mg/kg) 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 then inhibition percent was calculated.

General gross behavior and acute toxicity studies – Different doses (100-2000 mg/kg, p.o.) of CJP were administered to groups of 10 mice for 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 behavior changes, including: general motor activity, writhing, convulsions, response to tail pinching, gnawing, piloerection, pupil size, fecal output and feeding behavior and further up to 72 h for any mortality. Acute LD_{50} values in mice were calculated by the method of the Miller and Tainter [Miller *et al.*].

Statistical analysis – One-way analysis of variance (ANOVA) followed by Scheffe's test was applied for determining the statistical significance between different groups.

RESULTS

Carrageenin induced paw edema – Treatment with different doses of CJP (50 200 mg/kg) caused a significant ($P<0.05$ to $P<0.001$) and dose dependent inhibition of swelling caused by the λ carrageenin at 3 h equivalent to 26.82%-56.09% protection. Under same experimental condition the antiinflammatory effect of brufen was 63.41% at the dose of 100 mg/kg (Table 1).

Histamine-induced paw edema – CJP showed a significant ($P<0.01$ to $P<0.001$) and dose dependent antiinflammatory activity (15.79% to 57.89%). The effect was similar to the standard brufen and percent protection was 65.78% (Table 2).

Analgesy-meter induced pain – The experimental data of the force induced pain indicates that the CJP treated rats exhibited resistance against pain after 30 min. The pressure that indicates the pain after the treatment was significantly increased ($P<0.01$ to $P<0.001$) (Table 3).

Acetic acid induced writhing – Intraperitoneal administration of acetic acid (0.6%) induced pain reaction, which

Table 1. Effect of *C. indica* juice powder (CJP) on λ carrageenin induced edema in rats

Treatment	Dose (mg/kg)	Paw volume (ml) at 3 h	
		λ carrageenin	% Inhibition
Control	–	0.41±0.04	–
C J P	50	0.30±0.02[a]	26.82
C J P	100	0.22±0.02[b]	46.34
C J P	200	0.18±0.01[c]	56.09
Brufen	100	0.15±0.02[c]	63.41

Values are mean ± SEM for six rats.
P: [a]<0.05, [b]<0.01 and [c]<0.001 compared to control group.

Table 2. Effect of *C. indica* juice powder (CJP) on histamine induced edema in rats

Treatment	Dose (mg/kg)	Paw volume (ml) at 3h	
		Histamine	% Inhibition
Control	–	0.38±0.03	–
C J P	50	0.32±0.02	15.79
C J P	100	0.22±0.02[a]	42.11
C J P	200	0.16±0.02[b]	57.89
Brufen	100	0.13±0.02[b]	65.78

Values are mean ± SEM for six rats.
P: [a]< 0.01 and [b]< 0.001 compared to control group.

Table 3. Effect of *C. indica* juice powder (CJP) on force induced pain in mice

Treatment	Dose (mg/kg)	Weight causing pain (g)	
		Before administration	After administration
C J P	50	86.5±5.8	98.7±5.3
C J P	100	85.9±6.1	125.6±7.0[a]
C J P	200	88.4±5.5	149.2±6.5[b]
Brufen	100	84.7±5.7	161.1±7.9[b]

Values are mean ± SEM for six rats.
P: [a]< 0.01 and [b]< 0.001 compared to before administration group.

Table 4. Effect of C.indica juice powder (CJP) on acetic acid induced writhing in mice

Treatment	DOSE (mg/kg)	No of writhing	% Inhibition
Control	---	26.50±2.4	----
C J P	50	22.00±1.8	16.98
C J P	100	18.20±2.0[a]	31.32
C J P	200	17.10±1.6[b]	35.47
Brufen	100	16.78±1.7[b]	36.67

Values are mean ± SEM for six rats.
P: [a]< 0.05 and [b]< 0.01 compared to control group.

is characterized as a writhing response being 26.50±2.4. CJP given orally (50-200 mg/kg) significantly prevented the abdominal cramping at 100 and 200 mg/kg and the degree of percent inhibition was 16.98% to 35.47%, which is equivalent to that obtained by brufen at 100 mg/kg (Table 4).

General gross behavior and acute toxicity studies – CJP up to 2000 mg/kg body weight, orally, showed no gross evidence of any abnormalities or mortality in mice up to the end of the observation period.

DISCUSSION

CJP showed significant antiinflammatory activity against λ carrageenin and histamine induced acute paw edema in rats. Carrageenin is a sulphated polysaccharide obtained from seaweed (Rhodophyceae) is commonly used to induce acute inflammation and is believed to be biphasic. 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 the second phase of the edema is sensitive to most clinically effective antiinflammatory drugs, which has been used frequently to access the anti-edematous effect of natural products (Alcaraz *et al,* 1998; Della *et al.,* 1968). Prostaglandins play a major role in the development of the second phase of reaction, which is measured at around 3 h time (Di Rosa *et al.,* 1972). The carrageenin induced paw edema model in rats is known to be sensitive to cycloxygenase (COX) inhibitors and has been used to evaluate the effect of non steroidal antiinflammatory agents against which primarily inhibit the enzyme COX involved in prostaglandin synthesis. Based on these reports, it can be inferred that the inhibitory effect of CJP on carrageenin-induced inflammation in rats could be due to the inhibition of the enzyme cycloxygenase leading to inhibition of prostaglandin synthesis. But lipoxygenase inhibitors also posses significant antiinflammatory activity against carrageenin induced paw edema (Chawla *et al.,* 1987). So the inhibitory effect of CJP on carrageenin induced paw edema could also be due to its inhibitory effect on lipoxygenase pathway as the results are in accordance with Lino *et al.* (Lino *et al.*, 1997). Histamine is β imidazolylethylamine and has been implicated as a mediator of vasodilatation and other changes that occur during inflammation. The inhibitory effect of CJP on histamine induced paw edema gives an indication that the chemical constituents of CJP may be involved to maintain the normal circumference of acute injury.

The findings of this study have also furnished evidence that CJP prevent the pain reaction, which is characterized as writhing response. Constriction of abdomen, turning of trunk (twist) and extension of hind legs are taken as reaction to acetic acid induced writhing in mice. Moreover, significant resistance against mechanical pain indicates the potent analgesic activity of CJP.

It is also apparent from this study that CJP is well tolerated in mice after oral administration and does not

cause death or any toxic symptoms. 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 antiinflammatory and analgesic activities in modern clinical practice.

In conclusion, our results suggests that the administration of CJP showed inhibition of inflammation and pain in experimental animals. Further studies are in progress to find out the exact mechanism of action and the responsible active constituents.

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