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SHORT COMMUNICATION

Evaluation of anti-inflammatory and membrane stabilizing properties of ethanol extract of *Cansjera rheedii* J.Gmelin (Opiliaceae)

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ABSTRACT

Cansjera rheedii is widely used as a traditional medicine for the treatment and control of a variety of human inflammatory ailments by the fishermen of Auroville. In the present study, the anti-inflammatory and membrane stabilizing property of ethanol extract of *Cansjera rheedii* J. Gmelin (EECR) was evaluated in rat using the human red blood cells (HRBC) membrane stabilization method and carrageenin-induced acute paw edema model. The extract in concentration of 6-100µg/ml showed a dose-dependent inhibition of haemolysis of erythrocytes induced by hypotonic solution. Oral pre-treatment with EECR (250 mg/kg) inhibited carrageenin-induced paw edema (41.93%) within 3 hrs. It is concluded that the extract possess anti-inflammatory as well as membrane-stabilizing property.

Keywords: *Cansjera rheedii, Human red blood cell membrane stabilization, Carrageenin, Acute paw edema*

Cansjeera rheedii (Family-Opiliaceae) is a climbing shrubs, sometimes armed, commenly known as Kalimanakeerai in Tamil, is generally found in India through Malaya to Hong Kong and North Australia [1-2]. It has been used for the treatment of post-natal pain and for its hepatoprotective effects [3,4]. The fishermen of auroville were also using the plant for the treatment of inflammation. So, the present study was undertaken to establish scientific evidence for anti-inflammatory and membrane-stabilizing property of ethanol extract of *Cansjera rheedii* (EECR).

MATERIAL AND METHODS

Plant Material

The whole plants of Cansjera rheedii (Opiliaceae) were collected in and around Auroville, Puducherry, India in the month of June 2006 and it was identified and authenticated by Auro-Herbarium Sakthi Botanical Survey Department, Auroville. A voucher specimen has been kept in our laboratory for further reference (VS-12). The whole plant of Cansjera rheedii was cut into small pieces, shaded dried, powdered by a mechanical grinder and was passed through #40 mesh sieves and stored in an airtight container for further use.

Preparation of Extract

About 1 kg of the powdered plant material was successively extracted using ethanol (90%) in a soxhlet extraction apparatus. The extract was concentrated and traces of the solvent were completely removed under reduced pressure and stored in vacuum desiccators for further use. The extractw was named as ethanol extract of *Cansjera rheedii* (EECR). Preliminary qualitative chemical tests were carried out to find out the phytoconstituents present in it. The LD_{50} value of EECR was determined by using 2001OECD guidelines [5].

Animals

Adult male albino rats (Wistar Strain) weighing between 150-175g were obtained from Kings Institute, Chennai, India, and used for anti-inflammatory study. They were fed on commercial diet (Hindustan Lever) and water *ad libitum*. All the animals were acclimatized for a week before use. The room temperature was maintained at 25 ± 1ºC. The experimental protocol was approved by Institutional Animal Ethical Committee.

Chemicals

Diclofenac sodium, hyposaline, isosaline, phosphate buffer, alsever solution, carageenin and indomethacin

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Table 1:-Effect of ethanolic extract of *Cansjera rheedii* (EECR) on Human Erythrocyte Haemolysis

Drugs	% Prevention of lysis
EECR 100 µg/ml	36.14
EECR 50 µg/ml	33.31
EECR 25 µg/ml	21.05
EECR 12.5 µg/ml	19.52
EECR 6.3 µg/ml	14.28
Diclofenanc Sodium 100µg/ml	48.14

were purchased from Sigma Chemicals Co., St. Louis, USA.

EXPERIMENTAL DESIGN

Study of anti-inflammatory effects by membrane-stabilizing property:

Alsever solution prepared by 2% dextrose, 0.8% Sodium citrate, 0.05% citric acid and 0.42% sodium chloride dissolved in distilled water, then the solution was sterilized. Blood was collected from median cubital vein of healthy volunteers. The collected blood was mixed with equal volume of sterilized alsever solution. The blood was centrifuged at 3000 rpm and packed cells were washed with isosaline and a suspension in 10% (V/V) Isosaline was made. Various concentrations of the EECR were prepared in a mixture of 1ml Phosphate buffer, 2ml Hyposaline and 0.5ml HRBC suspension. Diclofenac sodium was used as the reference drug. Instead of hyposaline, 2ml of distilled water was used in control. The assay mixtures were incubated at 37ºC for 30 minutes and centrifuged. The haemoglobin content in the supernatant solution was estimated using UV analysis at 560nm. The percentage haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization or protection was calculated using this equation [6]:

Percentage inhibition of Haemolysis

$$= 100 \text{ X } OD_1\text{-}OD_2 / OD_1$$

Where $D_1$and OD_2 are absorbance of Diclofenac and EECR respectively.

Study of anti-inflammatory effects by Carrageenin-induced rat hind paw edema

The rats were divided into 3 groups comprising of six animals in each. Group 1 served as control and received 2 ml/kg of normal saline; Group 2 served as standard reference and received indomethacin (10 ml/kg), while Group 3 received EECR (250 mg /kg) by oral route. Edema was produced by the method described by Winter *et al* [7]. An injection of 0.1ml of 1% carrageen (in water) suspension into the right hid paw of each rat in the sub-plantar region was made. The paw volume was measured at 0 hour and 3 hours after injection of carrageenin. The plethysmograph apparatus was used for the measurement of rat paw volume based on Haris and Spencer method. Drug pretreatment was given 1 hour before the injection of carrageenin. The percentage of edema inhibition was calculated. All the results were analyzed for the significance using Students 't' test [8].

RESULTS AND DISCUSSION

The main action of anti-inflammatory agents is the inhibition of cyclooxygenase enzyme which is responsible for conversion of arachidonic acid to prostaglandins (PG). The main action of enzyme is conversion of prostaglandin G2 (PGG_2) to PGH_2 along with peroxidation which is associated with formation of long channels in membranes. The channel opening occurs due to release of chemical mediations and so arachidonic acid is released from membrane and converted to prostaglandin. The extracellular activity of these enzymes is said to be related to acute and chronic inflammation. Non-steroidal anti-inflammatory drugs (NSAIDs) act either by inhibiting these lysosomal enzymes (Cyclooxygenase) or by stabilizing the lysosomal membrane.

The extract at concentration range of 6-100 µg /ml protects the human erythrocyte membrane against lysis induced by hypotonic solution. At concentration of 100 µg /ml, the extract produced 36.14% inhibition of RBC haemolysis as compared with 48.14% produced by diclofenac sodium (Table 1). Since HRBC membranes are similar to lysosomal membrane components, the prevention of hypotonicity-induced HRBC membrane lysis was taken as a measure of anti-inflammatory activity of drugs. The results obtained demonstrate that EECR can significantly and dose-dependently inhibits RBC haemolysis (Table 1). It is well known that vital-

Table 2:- Effect of ethanolic extract of *Cansjera rheedii* (EECR) on carrageenin-induced paw edema

Treatment	Dose (mg/kg)	Increase in Paw volume (ml)	% Inhibition of edema
Normal Saline	-	0.62 ± 0.03	-
Indomethacin	10	0.24 ± 0.02	61.29
EECR	250	0.36 ± 0.04*	41.93

N=6, values are expressed as mean ± SEM

*p<0.001 when compared to control based on a Student's't' test

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ity of cells depends on the integrity of their membranes [9]. Exposure of RBC to injurious substances such as hypotonic medium, methyl salicylate or phenyl hydrazine results in the lysis of membrane accompanied by haemolysis and oxidation of haemoglobin [10-11]. The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Injury to RBC membrane will render the cell more susceptible to secondary damage through free radical-induced lipid peroxidation. This action is consistent with the observation that breakdown of bimolecules leads to the formation of free radicals which in turn enhance cellular damage [12-13]. The progression of bone destruction seen in rheumatoid arthritis is due to increased free radical activity [14-15]. Extract with membrane-stabilizing properties are well known for their interferring activity with the early phase of the inflammatory mediators release, namely the prevention of phospholipases release that trigger the formation of inflammatory mediators [16].

EECR (250 mg/kg, p.o.) also significantly inhibited carrageenin-induced rat paw edema ($p<0.001$). The inhibition at 3 hours was greater than at 1 hour after induction of edema (Table 2). Carrageenin-induced paw edema was taken as prototype of exudative phase of inflammation. The development of edema is biphasic [17]. The initial phase is attributable to the release of histamine, serotonin and kinins in the first hour after injection of carrageenin. A more pronounced second phase is related to the release of prostaglandins like substances in 2-3 hours. So, the possible anti-inflammatory activity of this extract may be due to inhibitory effect on release of inflammation mediators and/or by membrane stabilizing activity. From these findings, it is concluded that the ethanol extract of *Cansjera rheedii* possess membrane-stabilizing property as well as anti-inflammatory activity. Further works are in progress to find out its exact mechanism of action.

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