Analgesic and Antiinflammatory activity of the Chloroform Extract of *Trichilia connaroides* (W. & A.) Bentilizen

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The chloroform extract of dried leaves of *Trichilia connaroides*, was screened for analgesic and antiinflammatory activity, using chemical-, thermal- and formalin-induced inflammation in Swiss mice and Wistar rats. Chloroform extract showed significant and dose- dependent analgesic, and antiinflammatory activity.

Trichilia is a genus of trees, widely distributed in south east of Asia. *Trichilia connaroides* (W. & A.), Bentilezen, belonging to family Meliaceae, is known as *karai* or *karaivilangu* in Tamil. It is abundant in moist forests throughout the greater part of India. Phytochemical screening of leaves have been reported by several authors, like isolation of two tetracyclic triterpenoids having a 9,19-cyclopropane ring, and two new tetranoterpenoids-trijugin A and B, from chloroform extract[1-3]. The bark and leaves possess bitter and tonic properties[4]. Chloroform and methanol extract produced significant fall in cholesterol level within 24 hours, in Triton-induced hypercholesterolemia, when compared to control group[5], and also produced significant hypotensive effect in rats (unpublished data). However, literature review did not reveal screening for analgesic and antiinflammatory activities. The present study is to assess such activity of a chloroform extract using conventional animal models.

The leaves of *Trichilia connaroides* were collected from Salem, Tamilnadu during May/June 1998, and was identified at Botanical Survey of India, Pune, Maharashtra, where a voucher specimen has been deposited.

Formalin, n-hexane, chloroform, glacial acetic acid (S.D. Fine Chemicals, Mumbai), and dimethyl sulfoxide (Loba Chemie Pvt.Ltd, Mumbai), were of analytical grade. Aspirin, morphine sulfate, phenyl butazone, were gift samples from Government Drugs testing laboratory, Bangalore.

Swiss mice and Wistar rats used here, were maintained under standard animal house conditions, fed on commercial feed pellet, and water *ad libitum*. All experimental protocols were approved by IAEC.

The leaves of *T. connaroides* (2 kg) were shade-dried, and coarsely powdered. The powder was first defatted with n-hexane at room temperature. The powder was then extracted with chloroform, distilled, and last traces of solvent were removed by vacuum (yield: 30 g).

The coarse powder of leaves (500 g) was subjected to successive extraction with different solvents in the increasing order of polarity, from hexane, benzene, chloroform, and mixture of chloroform and ethyl acetate. The dry extracts were subjected to various chemical tests to detect the presence of different phytoconstituents[6,7].

Healthy Swiss mice of either sex, weighing around 25-30 g, and overnight- fasted, were used for study. Food was withdrawn during the study, however free access to water was provided. The weighed animals were randomly assigned to six groups of six animals each (n=6), and were administered the extract orally, in the increasing doses 10, 30, 100, 300 and 1000 mg/kg. No adverse effect or mortality was detected up to 500 mg/kg, during the 24

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h observation period. Further, chloroform extract was screened for analgesic and antiinflammatory activities.

Antiinflammatory studies[8] were carried out in formalin-induced paw edema in Wistar rats. Overnight-fasted male Wistar rats were randomly assigned to five groups (n=6). Such animals received orally solvent, phenylbutazone, or the chloroform extract, as shown in the Table 1. Inflammation was produced in these animals by injection of 0.1 ml of 1% w/v formalin into the subplantar region of left hind paw. The paw volume was measured using mercury displacement technique, with the help of plethysmograph at 0 and 3 h after formalin injection. The difference between 0 and 3 h reading were taken as the volume of edema and percentage of edema inhibition, and was calculated for each group. The results are summarised as shown in Table 1.

In Acetic acid-induced writhing test[9], the prescreened Swiss mice were assigned into six groups, each containing six animal (n=6). The extract was dissolved in 50% DMSO, and was administered subcutaneously. Writhing was induced 30 min later, by intraperitoneal injection of 0.1 ml of 0.6% acetic acid. The number of writhes were counted for 30 min, immediately after acetic acid injection, in all animals. Percentage protection was calculated for all groups. Aspirin 100 mg/kg was used as standard drug.

In the thermal method[10], the prescreened Swiss mice (reaction time: 3-4 sec) were assigned randomly into six groups of six animals each (n=6). The extract was dissolved in 50% DMSO, and was administered intraperitoneally. The delay in reaction time (hind paw licking/jump response) of animals, when placed on hot plate maintained at 55°±0.1° (Eddy's analgesiometer, INCO), was recorded and tabulated. A cut-off time was fixed at 15 sec, to avoid damage to the paws. Morphine sulfate, 5 mg/kg was used as standard analgesic.

Preliminary phytochemical screening revealed the presence of flavanoid, glycoside, steroids, saponins, and phenolic compounds. Neither toxicity signs nor behavioural changes observed up to 500 mg/kg. Chloroform extract in the dose 60 and 90 mg/kg produced dose-dependent, statistically significant ($P<0.05$), antiinflammatory reaction in the chosen model, and was comparable to inhibition produced by phenylbutazone (Table 1).

Chloroform extract in the dose of 60 and 90 mg/kg, significantly ($P<0.05$) suppressed acetic acid writhing response in animals, in a dose-dependent manner. The percentage protection is tabulated as shown in Table 1. Chloroform extract in the doses of 60 and 90 mg/kg body weight, increased reaction time in a dose-dependent manner. The results were statistically significant ($P<0.05$).

The rat formalin test which causes a local injury of the paw, is used as a model for tonic pain[11], and localized inflammatory pain[12,13]. There are two phases of responses, while the stimulus during the early phase is a direct chemical stimulation of nociceptors, and that during the late phase, involves inflammation[14]. Formalin-induced pain is caused primarily by peripheral tissue inflammation[15]. A central sensitization of dorsal horn neuron occurs during inflammatory pain. Acute inflammation may last for relatively shorter duration, ranging from few minutes to few days. Exudation of fluid and plasma proteins, emigration of leukocytes, and predominantly neutrophils, are characteristic changes[16].

The abdominal contraction response induced by acetic acid, is a sensitive procedure to establish peripherally acting analgesics, and such a response is thought to involve local peritoneal receptors. Significant protection was observed in extract (60 and 90 mg/kg) treated group of animals, and is roughly comparable to standard drug.

In the thermal method, the extract-treated animals (60 and 90 mg/kg) exhibited statistically significant elevation in

TABLE 1: ANTIINFLAMMATORY AND ANALGESIC EFFECT OF CHLOROFORM EXTRACT OF *T. CONNAROIDES*

Group	Dose (mg/kg)	Antiinflammatory activity		Analgesic activity	
		Increase in paw volume (ml)	Percentage inhibition of paw edema	No. of writhes	Percentage protection
Control 50% DMSO	2 ml/kg	0.93 ± 0.25	-	24.2 ± 0.97	-
Phenylbutazone	150	0.23 ± 0.03	75.7 ± 2.04*	-	-
Aspirin	100	-	-	11.3 ± 1.06	53.1 ± 1.46
Chloroform extract	30	0.75 ± 0.05	19.0 ± 3.23*	23.5 ± 0.01	3.74 ± 0.94
Chloroform extract	60	0.73 ± 0.05	21.6 ± 0.58*	14.7 ± 1.02	39.9 ± 0.54
Chloroform extract	90	48.5 ± 4.09	48.5 ± 4.09*	12.8 ± 1.17	48.5 ± 1.52

Values are mean ± SE of six animals in each group, *$P<0.05$ when compared to control

TABLE 2: ANALGESIC ACTIVITY OF CHLOROFORM EXTRACT OF *T. CONNAROIDES* IN THERMAL METHOD

Group	Dose (mg/kg)	Mean basal reaction time (in secs)	Percentage increase in basal reaction time
Solvent control	2 ml/kg	3.22±0.57	-
Morphine sulfate	5	11.0±0.63	70.6*
Chloroform extract	30	4.60±0.6	29.8
Chloroform extract	60	8.50±0.5	62.0*
Chloroform extract	90	9.35±0.35	65.5*

Values are expressed as mean±SE of six animals, *P<0.05 when compared to control

mean basal reaction time. Effect of 90 mg/kg chloroform extract is almost comparable to that of standard drug. This points to the involvement of higher centres in the analgesic activity. The chloroform extract in the doses 60 and 90 mg/kg, significantly suppressed formalin-induced paw edema. However, it was less effective than standard drug, phenylbutazone.

Analgesic activity of extract was significant in acetic acid-induced writhing model than in the thermal method (P<0.05) (Table 2), thus it appears that chloroform extract inhibits predominantly peripheral pain mechanism.

Preliminary phytochemical screening of chloroform extract of *T. connaroides* revealed presence of flavanoid compounds. Flavonoids are known to target prostaglandins, which are involved in the late phase of acute inflammation and pain perception.[17] In the light of the above findings, we presume that the presence of flavonoids may be contributing to analgesic and antiinflammatory activities of chloroform extract of *T. connaroides*. Further study may reveal the exact mechanism of analgesic and antiinflammatory activities of the chloroform extract.

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