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Analgesic activity of *Cuscuta campestris* Yuncker a parasitic plant grown on *Nerium indicum* Mill

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ABSTRACT:

Cuscuta campestris is the most widespread species in the genus *Cuscuta* belonging to family Convolvulaceae throughout the World. *Cuscuta campestris* possess to have anthelmintic activity. The ethanol extract of plant also proved to have Analgesic, anti-pyretic and anti- inflammatory activity (Azza, *et al.,* 1996) on different host. The dried methanol extract (CC ME) of the whole plant of *Cuscuta campestris* Yuncker was studied for its analgesic activity grown on *Nerium indicum* (Apocyanaceae). Analgesic activity was carried out using animal models i.e. Acetic acid Induced Writhing Test (Chemical Stimulation) and Heat conduction method in comparison with Diclofenac sodium as reference standard (Positive control). The CC ME was given in doses of 100, 200 and 400 mg/kg body weight in which dose 400 mg/kg given significant results in comparison with reference standard Diclofenac sodium. The study may conclude that some chemical entities transferred from host *Nerium indicum* to the parasite *Cuscuta campestris* are responsible for analgesia.

Key words: *Cuscuta campestris* Yuncker, analgesic, whithing, heat conduction.

INTRODUCTION:

The genus *Cuscuta* L. (dodder) contains 180 species [15, 13], distributed throughout the World mostly in tropical and subtropical regions of the world. *Cuscuta campestris* is the most widespread species in the genus *Cuscuta* belonging to family Convolvulaceae throughout the World. This parasitic weed is native from North America which has spread to the Old World [5]. All the resources required to it is obtains from its host plants and severely suppressing them which may cause death of host plant [2, 4, 5]. *C. campestris* is among the most damaging parasite due to its wide geographical distribution and host range worldwide causing. *C. campestris* cause severe damages to carrots, alfalfa, sugarbeet, onions, legumes and other crops [14, 5, 8]. Unlike root

parasites, *C. campestris* seeds do not require a specific stimulant to induce germination. Mechanical or chemical scarification of the seed coat is sufficient to facilitate germination [9, 5]. *Cuscuta campestris* possess to have anthelmintic activity [10]. The plant also proved to have Analgesic, anti-pyretic and anti- inflammatory activity [3] on different host. Due to having adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all the cases. Therefore, analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant based drugs [12]. Present study attracts analgesic activity of *Cuscuta campestris* due to adverse effect of allopathic analgesics.

MATERIALS AND METHODS:

Plant material:

The plant material of *Cuscuta campestris* Yuncker (Convolvulaceae) parasitic on *Nerium indicum* Mill. (Apocyanaceae) was collected from Baroda- Ahmedabad Express way of Gujarat (16° 41' 60N Latitude and 74° 13' 0E Longitude and 300m Altitude) in December 2010. The plant specimen was submitted to museum of Department of Pharmacognosy, Sigma Institute of Pharmacy, Baroda, Gujarat, India. The voucher specimen no. is RSG2412.

Preparation of extract:

The shade dried material was coarsely powdered (500 g) and extracted with methanol (1.5 L) by cold maceration method. The extract (CC ME) was filtered, concentrated on rotary vacuum evaporator and further dried in vacuum dryer and weighed (11.23% w/w). Dosages of different concentrations of extract were dispensed from solution prepared from normal saline. The vehicle alone served as control.

Animals:

Swiss Albino mice (20-25 g) were procured from SPARC, Baroda. The animals were housed under standard conditions of temperature (22 ± 1^0C), relative humidity (55 ± 10%), 12 hr light/dark cycles and fed with standard pellet diet (Amrut, Pranav Agro Industries Ltd., Sangli, India) and water *ad libitum*. All the experiments performed are approved by the Institutional Animal Ethical Committee (SIP/IAEC/2010-11/06).

Grouping of animals:

The animals were divided into six groups each containing six animals. Group I served as untreated control and received normal saline (3 ml/kg, p.o.), group II served as positive control and received Diclofenac sodium (5 mg/kg, i.p.) and group III, IV and V were treated with 100, 200, and 400 mg/kg of CC ME respectively.

Acute toxicity test:

The acute toxicity of *Cuscuta campestris* methanol extract was determined in Swiss Albino mice according to the method [7]. with slight modifications. Mices fasted for 16 h were randomly divided into groups of six rats per group. Graded doses of the extract (250, 500, 1000 and 2000 mg/kg p.o.) were separately administered to the mices in each of the groups by means of bulbed steel needle. All the animals were then allowed free access to food and water and observed over a period of 48 h for signs of acute toxicity. The number of deaths within this period was recorded.

Analgesic activity:

1. Acetic acid Induced Writhing Test (Chemical Stimulation):

The analgesic activity of the samples was evaluated using acetic acid induced writhing method in mice [1]. In this method, acetic acid is administered intraperitoneally to the experimental animals to create pain sensation. As a positive control, any standard NSAID drug can be used. In the present study Diclofenac sodium was used to serve the purpose. The CC ME was administered orally in three different doses (100, 200 and 200 mg/kg body weight) to the Swiss Albino mice after an overnight fast. Test samples and vehicle were administered orally 30 minutes prior to intraperitoneal administration of 0.7% v/v acetic acid solution (0.1ml/10g) but Diclofenac sodium was administered 15 minutes prior to acetic acid injection. Then the animals were placed on an observation table. Each mouse of all groups were observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group while Diclofenac sodium (5 mg/kg) was used as a reference standard (positive control)

2. Heat conduction method:

The animals were divided into five groups of 6 animals each. Group I served as negative control. Group II served as positive control and were injected Diclofenac sodium (5 mg/kg) intraperitonially. Group III, IV and V were treated with 100, 200, and 400 mg/kg body weight of CC ME respectively. After half hour, 1 hour, 2 hour and 3 hour, the tip of tail was dipped up to 5 cm into hot water maintained at 58°C. The response time was noted as the sudden withdrawal of the tail from the hot water. Cut off time of 10 seconds was maintained to avoid damage to the tail for all groups. The time required for flicking of the tail, was recorded, to assess response to noxious stimulus [11].

Statistical analysis:

All the values ware statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test. Comparison between control and drug treated groups were considered to be significant. All values are expressed as mean ± SEM.

RESULTS AND DISCUSSION:

Oral administration of graded doses (250, 500, 1000 and 2000 mg/kg p.o.) of the methanol extract of *Cuscuta campestris* to mices produce no significant changes in behavior. No mortality was however recorded for all the doses after 72 h of administering the extract to the animal.

Table 1. Analgesic effect of methanol extract of *Cuscuta campestris* on Swiss albino mice's using Heat conduction method

Treatment	**Dose (mg/kg)**	**Reaction Time in Seconds (Mean ± SEM)**			
	0 hr	0 hr	1 hr	2 hr	3 hr
Negative Control (Normal Saline)	5 ml/kg	4.47 ± 0.13	5.14 ± 0.17	4.23 ± 0.15	4.49 ± 0.19
Positive Control (Diclofenac sodium)	5 mg/kg	4.28 ± 0.10	11.34±0.34**	8.23±0.24**	9.33 ± 0.14 **
CC ME	100 mg/kg	4.81 ± 0.14	5.93 ± 0.24	5.10 ± 0.11	6.24 ± 0.17*
CC ME	200 mg/kg	4. 15 ± 0.14	5.98 ±0. 24	7.15 ± 0.17*	7.01 ± 0.12*
CC ME	400 mg/kg	4. 12 ± 0. 17	7.13 ± 0.18 *	7.11 ± 0.23 *	8.13 ± 0.17 **

Values are expressed as Mean ± SEM, n=6, Data analyzed by One-way ANOVA followed by Dunnette's test ** P < 0.01, * P < 0.05, CC ME (*Cuscuta campestris* methanol extract).

Table 2. Analgesic effect of methanol extract of *Cuscuta campestris* on Swiss albino mices using Acetic Acid Induced Writhing method.Acetic Acid Induced Writhing

Treatment	Dose	Number of Writhes observed	Percentage Inhibition
Negative Control (Normal Saline)	5 mL/kg	34.8 ±1.69	--
Positive Control (Diclofenac sodium)	5 mg/kg	10.7 ± 0.98**	67.38
CC ME	100 mg/kg	29.3 ± 0.94*	15.81
CC ME	200 mg/kg	24.0 ± 0.92*	26.83
CC ME	400 mg/kg	18.2 ± 0.69**	44.51

Values are expressed as Mean ± SEM, n=6, Data analyzed by One-way ANOVA followed by Dunnette's test ** P < 0.01, * P < 0.05, CC ME (*Cuscuta campestris* methanol extract).

Acetic acid induced writhing in mice attributed visceral pain finds much attention of screening analgesic drugs [6]. The crude extract the plant showed significant analgesic action compared to the reference drug Diclofenac sodium against acetic acid induced pain in mice and heat conduction method at three dose levels i.e. 100, 200 and 400 mg/kg b. wt. The results of present study indicate the methanol extract of *Cuscuta campestris* possesses analgesic effect. Analgesic effect of the extracts was demonstrated in the experimental models using Acetic acid Induced Writhing Test (Chemical Stimulation) and Heat conduction method using thermal stimuli. An increase in reaction time and decrease in writhing are generally considered an important parameter of analgesic activity in Heat conduction method and Acetic acid Induced Writhing Test respectively. *Cuscuta campestris* is feeding on its host; in the present study *Cuscuta campestris* is parasitic on *Nerium indicum.* So, it may conclude that chemical constituents of *Nerium indicum* are responsible for analgesic activity of *Cuscuta campestris* due to transfer of them from host to parasite [16].

CONCLUSION:

The methanol extract of *Cuscuta campestris* showed significant results for analgesic activity. As the holo- parasite the plant get feeds on its host plant. Many of the primary as well as secondary metabolites may transfer from host to parasite. The analgesic effect of *Cuscuta campestris* may be due to the metabolites transfer from *Nerium indicum*. The present study may conclude that pharmacological activities of parasitic plants are depending on their hosts. If same parasite grown on different hosts then it may having different pharmacological actions or same pharmacological action with varying results.

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