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EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY PROPERTIES OF *CHLORIS BARBATA* (SW.)

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Abstract

The petroleum ether extract of *Chloris barbata* (SW.) was evaluated for analgesic and anti-inflammatory activity at the doses of 200 and 400 mg/kg body weight. The tail immersion and acetic acid writhing response in mice were used to assess analgesic activity. The acute toxicity study of the extract had shown no sign of toxicity up to a dose level of 2000mg/kg body weight. Carrageenan induced paw edema in rats, which is an acute model used to assess anti-inflammatory activity. The extract has inhibited paw edema in dose related manner. A dose dependent analgesic action was obtained against tail immersion and writhing test indicating that analgesic activity may be involved in the inhibition of the pain. Thus the extract of *Chloris barbata* (SW.) possesses significant analgesic and anti-inflammatory activities.

KEY WORDS: *Chloris barbata* (SW.), anti-inflammatory, analgesic, writhing

INTRODUCTION

Inflammation is a defense mechanism and protective response of vascular tissues to harmful stimuli like pathogens, damaged cells, allergens, irritants (Ferrero-Miliani *et al.,* 2007). Inflammation is of two types i.e. acute and chronic. 'Acute inflammation' is the initial response of harmful stimuli appears within a few minutes or hours, done by increased movement of plasma and leukocytes from blood to injured tissues. Algesia (pain) is an ill defined, unpleasant sensation occurs by external or internal noxious stimuli. Pain is a warning signal which is discomfort, excessive pain is unbearable and leads to sinking sensation, sweating, nausea, rise or fall in BP, tachypnoea (Tripathi, 2003). Many synthetic drugs were now available in market to treat inflammation and pain, leading to side effects. So, the herbal drugs of the utmost important and there is a need for the production of novel herbal drugs.

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Chloris barbata (SW.), commonly called as swollen finger grass (Family: poaceae) is a tufted annual grass about 70cm high, internodes are longer at the top and shorter at base; leaves lanceolate, narrowly linear, acuminate; spikes 6cm long, floral glumes densely hair, awned, grains oblong. Frequently found along cultivated fields and in forest hilly areas (Pullaiah 1963; Rolla Seshagiri Rao, 1920). The whole plant is used in treating Rheumatism (Madhava Chetty *et al.,* 2008). The juice from the plant is used in treating various skin disorders and possesses anti-diabetic, antimicrobial properties (Algesaboopathi, 2009). Traditionally *Chloris barbata* (SW.) has been used in treatment of many types of pain and inflammatory conditions, no scientific report is available and so the present study has been carried out to investigate the analgesic and anti-inflammatory properties of petroleum ether extract of *Chloris barbata* (SW.)

MATERIALS AND METHODS

Plant material

The whole plant of *Chloris barbata* (SW.) was collected from the cultivated fields of chandragiri, chittoor district of Andhra Pradesh, India. The plant was

authenticated prof. P. Jayaraman, Director of National Institute of Herbal Science, W. Tambaram, Chennai. The voucher specimen (PARC/2009/352) of the plant was deposited at the college for further reference.

Preparation of Extract

The whole plant *Chloris barbata* (SW.) was dried in shade and pulverized in grinder-mixer to obtain a coarse powder, then passed through the 40 mesh sieve. A weighed quantity (85gm) of powder was subjected to continuous hot extraction with petroleum ether in soxhlet apparatus for 48 hours. Then the extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. The percentage yield of petroleum ether extract of *Chloris barbata* (SW.) was found to be 2.94%w/w

Animals used

Adult albino mice (30-40gm) and Adult albino rats (150-200gm) were obtained from the animal house in Sree Vidyaniketan College of pharmacy, Tirupathi, Andhra Pradesh. The animals were maintained in a well ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan lever limited, Bangalore) and water was given ad libitum. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref. No. /AEC/XIII/05/SVCP/2008-09.

Acute Toxicity Study

The acute toxicity PECB was determined as per the OECD guideline no. 423 (Acute toxic class method). It was observed that the test extract was not mortal even at 2000mg/kg dose. So, $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of the dose were selected for further study (OECD, 2002).

Analgesic study

Writhing test

Abdominal constriction induced by intraperitoneal injection of acetic acid was carried out by method of Koster *et al.,* (Koster *et al.,* 1959). *Chloris barbata* (SW.) extract was tested at 200 and 400 mg/kg, p.o and Indomethacin (10 mg/kg, p.o) a reference analgesic and anti-inflammatory drug, here the writhing inhibition produced by the plant extract was determined by comparing the reference drug. Intraperitoneal injection of acetic acid (0.7%) at a dose of 0.1 ml/10g of body weight was used to create pain sensation. The number of writhings was calculated for 10 min, 10 min after the application of acetic acid.

Tail Immersion test

The basal reaction time to radiant heat by placing the tip of the tail in a beaker of water maintained at 55°C was carried out. The *Chloris barbata* (SW.) extract was tested at 200 and 400 mg/kg, p.o and compared to the reference compound Indomethacin 10 mg/kg. p.o. Tail withdrawal is taken as the end point, a cut off point of 15 sec is observed to prevent the damage to the tail. The percent increase in reaction time at each time interval was calculated.

Anti-Inflammatory Study

Carrageenan – Induced rat paw edema

Anti-Inflammatory activity was evaluated using the carrangeenan induced rat paw edema (Winter *et al.,* 1957). The *Chloris barbata* (SW.) extract was tested at 200 and 400 mg/kg, p.o and indomethacin 10mg/kg, p.o is a reference compound. The inhibition of the edema produced by the plant drug is compared to the reference drug. After one hour of the treatment of the drugs, 0.1 ml of 1% w/v carrageenan suspension was injected subcutaneously into the plantar surface of the right hind paw. The paw volume was measured by using plethysmometer up to 3 hours after carrageenan injection.

$$\text{Percentage edema} = \frac{V_t - V_o}{V_o}$$

V_t = Volumes for each group

V_o = Volume obtained for each group before any treatment

Percentage inhibition of edema =

$$\frac{(V_o - V_t)\text{Control} - (V_t - V_o)\text{treated}}{(V_o - V_t)\text{Control}} \times 100$$

Statistical Analysis

The data were expressed as mean ± standard error mean (S.E.M). The significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnett's test, p values less than 0.05 were considered as significance.

RESULTS

Analgesic activity

Writhing test

In case of the acetic acid writhing test, at doses of 200 and 400 mg/kg the PECB inhibited the writhing responses and the number of writhes were significantly lower than the control group and the maximum inhibition is seen at 400 mg i.e., 60.69%. Indomethacin has produced as protective effect and exhibited 72.64% of inhibition at a dose of 10 mg/kg. (**Table 1**)

Tail immersion test

The PECB at the doses of 200 and 400 mg/kg produced significant delay in response of tail withdrawal compared to control and it was higher at 400 mg/kg and the delay in response was higher by indomethacin at a dose of 10 mg/kg. **(Table 2)**

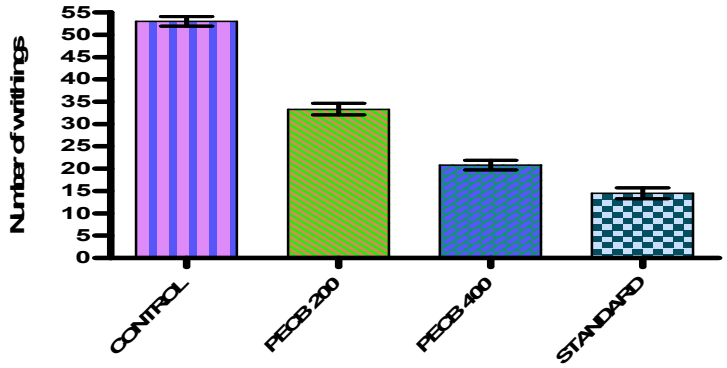
Anti-inflammatory study

The percentage inhibition of edema values of carrageenan induced rat paw edema in the table 3 was given. The PECB in the doses of 200 mg and 400 mg/kg body weight showed 63.69% and 70.83%. Inhibition of edema and at 400 mg dose the inhibition was higher, how ever the indomethacin 10 mg/kg has exhibited a protective effect and the percentage inhibition of edema was 76.19%. **(Table 3)**

Table 1. Analgesic effect of petroleum ether extract of C*hloris barbata* (SW.) (PECB) in acetic-acid induced writhing test

Group	Design of treatment	Number of writhings	% inhibition
I	Control (Normal saline, 10 ml/kg)	53 ± 1.06	---
II	PECB (200mg/kg bw, p.o)	33.33 ± 1.28***	37.11
III	PECB (400 mg/kg bw, p.o)	20.83 ± 1.10***	60.69
IV	Indomethacin (10 mg/kg bw, p.o)	14.5 ± 1.25***	72.64

n=6, values are expressed as mean ± SEM, P < 0.05 when compared with control



Analgesic effect of Chloris barbata (SW.) in acetic acid induced writhing test

Table 2. Analgesic effect of petroleum ether extract of *Chloris barbata* (SW.) (PECB) in tail immersion method.

Group	Design of treatment	Tail withdrawal in sec					
		0 min	**15 mins**	**30 mins**	**60 mins**	**120 mins**	**180 min**
I	Control (Normal saline, 10 ml/kg, p.o)	2.66 ± 0.21	3 ± 0.36	2.66 ± 0.33	2.83 ± 0.30	3.16 ± 0.31	2.83 ± 0.31
II	PECB (200mg/kg bw, p.o)	3.16 ± 0.30	4.5 ± 0.43	5.16 ± 0.48**	4.83 ± 0.30**	5.66 ± 0.33**	5.55 ± 0.50**
III	PECB (400 mg/kg bw, p.o)	3.16 ± 0.30	4.66 ± 0.42	5.5 ± 0.43***	5.16 ± 0.31***	6.33 ± 0.33***	7.3 ± 0.56***
IV	Indomethacin (10 mg/kg bw, p.o)	3.5 ± 0.22	5.16 ± 0.54*	6.5 ± 0.43***	6.5 ± 0.34***	7.83 ± 0.54***	9.16 ± 0.60***

n=6, values are expressed as mean ± SEM, P < 0.05 when compared with control.

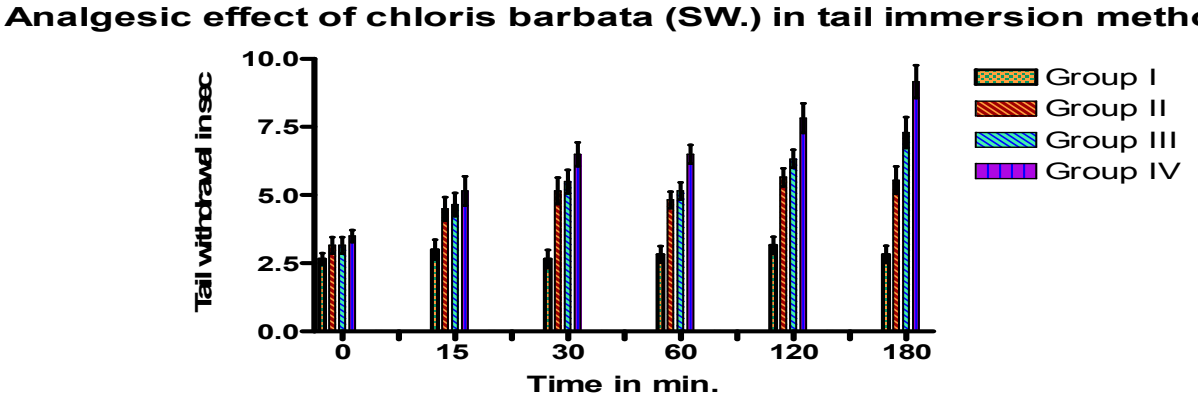
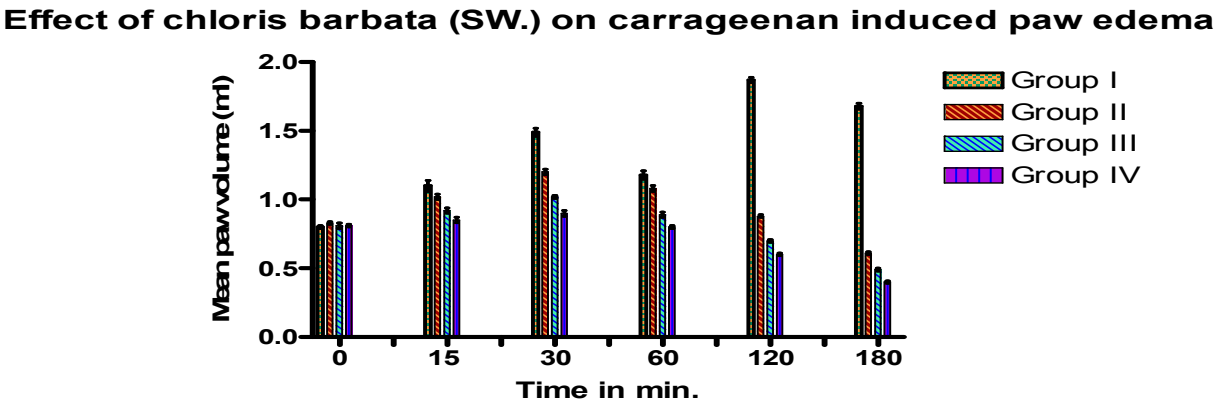


Table 3. Effect of C*hloris barbata* (SW.) on carrageenan induced paw edema

Group	Design of treatment	Mean paw volume (ml)						% inhibition after 180 mins
		0 min	15 mins	30 mins	60 mins	120 mins	180 mins	
I	Control (Normal saline, 10 ml/kg)	0.80 ± 0.01	1.10 ± 0.04	1.49 ± 0.03	1.18 ± 0.03	1.87 ± 0.02	1.68 ± 0.02	---
II	PECB (200mg/kg bw, p.o)	0.83 ± 0.01	1.02 ± 0.02	1.20 ± 0.02***	1.08 ± 0.02***	0.88 ± 0.01***	0.61 ± 0.01***	63.69
III	PECB (400 mg/kg bw, p.o)	0.81 ± 0.02	0.92 ± 0.02***	1.02 ± 0.01	0.89 ± 0.02***	0.70 ± 0.01***	0.49 ± 0.01***	70.83
IV	Indomethacin (10 mg/kg bw, p.o)	0.81 ± 0.01	0.85 ± 0.02***	0.9 ± 0.02***	0.80 ± 0.01***	0.6 ± 0.01***	0.40 ± 0.01***	76.19

n=6, values are expressed as mean ± SEM, P < 0.05 when compared with control.



DISCUSSION

In the present study the potential analgesic and Anti-inflammatory effect of the petroleum ether extract of *Chloris barbata* (SW.) was investigated. The results indicate that the oral administration of PECB exhibit a significant and dose dependent protective effect on chemical (acetic acid injection) and thermic (heat) painful stimuli at the doses of 200 and 400 mg/kg and indicates that PECB possess both peripheral (writhe reduction) and central (prolongation of tail withdrawal) effects.

Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1–2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissues surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (Brito and Antonio, 1998). The inhibitory activity shown by the extract of *Chloris barbata* (SW.) (400 mg/kg, p.o) over a period of 3h in carrageenan-induced paw inflammation was quite similar to that exhibited by the group treated with indomethacin. These results indicate that it acts in later phases, probably involving arachidonic acid metabolites, which produce an edema dependent on neutrophils mobilization (Just *et al*., 1998). Thus, the results of the study would support the traditional use of *Chloris barbata* (SW.) in some painful and inflammatory conditions.

The intraperitoneal administration of agent acetic acid that irritate serous membranes provokes a stereotypical behavior in mice and rats which is characterized by abdominal contractions, movements of the body as a whole, twisting of dorsoabdominal muscles, and a reduction in motor activity and coordination (Bars *et al*., 2001). The quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of rats obtained after the intraperitoneal injection of acetic acid demonstrated high levels of prostaglandins $PGE_{2\alpha}$ and $PGF_{2\alpha}$ during 30 min after stimulus (Deraedt *et al*., 1980). It should be taken into consideration that the mechanism involved in the genesis of the carrageenan-induced edema can cause the release of prostaglandins and kinins, among other substances (Garcia-Leme *et al*., 1973). The writhing test has shown results similar to that obtained in the edematogenic assay using carrageenan.

On the other hand, the lack of influence of extracts of *Chloris barbata* (SW.) on the reaction time of mice submitted to the tail immersion is consistent with the interpretation that its analgesic property does not have a central origin, having an analgesic effect in the acetic acid writhing test that is mostly mediated via a peripheral mechanism by interfering with the local reaction caused by the irritant or by inhibiting the synthesis, release and/or antagonising the action of pain mediators at the target sites (Srinivasan *et al*., 2003).

Finally, the results of the present study confirm that *Chloris barbata* (SW.) has analgesic and anti-inflammatory activities. Therefore, the native practioners using this plant for treatment of pain and fever. There is a need for further studies in order to isolate the active ingredients in the plant that is responsible for its biological activities and to elucidate the mechanism of action of these active ingredients.

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