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ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF *COLDENIA PROCUMBENS* LINN

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ABSTRACT: The methanolic extract of whole plant *Coldenia procumbens* was screened for its analgesic activity. The crude extract was assessed for central analgesic effect using *in-vivo* methods like hot plate method, tail-flick method, and tail-immersion method at two different doses. The extract at the dose of 200mg/kg body weight showed significant ($p<0.001$) analgesic effect comparable to the standard drug Pentazocine at the dose of 10mg/kg body weight. The peripheral analgesic was assessed by acetic acid-induced writhing test in mice. The methanolic extract at a dose of 200 & 400mg/kg body weight found to show significant ($p< 0.001$) analgesic effect comparable to the standard drug Aspirin at the dose of 20mg/kg body weight.

INTRODUCTION: [1-6] *Coldenia procumbens* is a Procumbent deep rooted, hairy herb wide spread in tropical and sub tropical Africa, Asia and Australia, found throughout India as a weed in moist place. It is often found in seasonally flooded locations, e.g. on dry rice fields, where it is a common weed, but it can also withstand severe drought. It used as external application for causing suppuration of boils. In folklore medicine it is used to treat rheumatic swellings, leucorrhoea, menorrhagia, anti-diabetic, anti-arthritic and hypotensive. Pain is associated with a wide range of injury and disease, and is sometimes the disease itself. Some conditions may have pain and associated symptoms arising from a discrete cause, such as post operative pain or pain associated with a malignancy, or may be conditions in which pain constitutes the primary problem, such as neuropathic pains or headaches.

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In view of traditional uses of Coldenia and the Phytochemical review [1] & Pharmacological activities like anti inflammatory [2, 3] hepatoprotective [4], antibacterial [5] & anthelminthic [6] activities reported on this plant the present study has been undertaken to investigate the analgesic activity of methanolic extract of *Coldenia procumbens*.

MATERIALS AND METHODS:

Plant materials:

The whole plant of *Coldenia procumbens*. (Family: Boraginaceae) was collected at Warangal District, A.P, India. The plant was identified and authenticated by Prof. Prathibha Devi, HOD-Department of Botany, Osmania University, Hyderabad. The given voucher specimen number 0468 and specimen was deposited at botany department of Osmania University for future reference.

Preparation of the extract:

The whole plant was dried in shade at room temperature and made to coarse powder and Extracted using Methanol by simple maceration technique for seven days. The solvent recovery and

concentration of extract was done by using Rotary flash evaporator (Heidolph). The obtained crude extract was stored in air tight container in refrigerator below 10°c for further studies.

Animals: Experimental animals:

Swiss Mice weighing between 20-25g were used in this investigation. Animals were purchased from the Teena Biolabs Pvt. Ltd, Hyderabad. All the animals were kept for acclimatization for 2weeks under laboratory conditions and fed with pellet diet and tap water ad libitum.

Acute toxicity studies:

Toxicity studies were conducted as per OECD Guidelines. No mortality observed up to 2000mg/kg, for methanol extract, so this dose was considered as the maximum tolerated dose and as per the guidelines the two doses 200 mg/Kg b. wt and 400 mg/Kg b. wt were selected for the study.

***In-vivo* Analgesic methods[7-11.]**

Hot plate method:

This test was performed according to the method described in S.K. Kulkarni, Hand book of phamacology, 2010, with slight modifications. Male Swiss mice weighing 25-30 g were divided in to 4 groups of 6 animals in each group. The hot plate was maintained at 56±1°C. Animals were placed into a glass cylinder of 24cm diameter on the heated surface & the time between placement & licking the paws or jumping was recorded as response latency. The reaction time was recorded for control mice, for animals treated with pentazocine (10mg/kg I.p) and the extract treated group at 30, 60 & 120min. The test was terminated at 15sec to prevent tissue damage.

Tail-flick method:

This test was performed according to the method described in S.K. Kulkarni, Hand book of phamacology, 2010, for quantitative measurements of pain threshold against thermal radiation and for evaluation of analgesic activity in mice. The mice were weighed and numbered properly and exposed to radiant heat by placing the hip of the tail. Flicking response was taken as an end point; normally a mouse withdraws its tail within 3-5sec. A cut off period of 10-12sec was observed to prevent damage of the tail. Any animal failing to withdraw its tail in 3-5sec was rejected from the study. At least 3-5basal reaction times for mice were taken at a gap of 15min to confirm normal behavior of animal.

Tail - immersion test:

This test was performed according to the method described in S.K. Kulkarni, Hand book of pharmacology, 2010. Prior to analgesic experiments, the animals were screened for the sensitivity test by immersing the tail of the mice gently in hot water maintained at 55°C-55.5°C. The animal immersing the tail from hot water with in 5 sec was selected for the study. The selected mice were then divided into four groups of six mice each. Group- 3 & Group – 4 received the extract in 2%w/v Gum acacia in normal saline intraperitoneally at a dose of 200mg/kg & 400 mg/kg respectively.Group-2 received pentazocine (10mg/kg) & Group-1 received 2% w/v of Gum acacia in normal saline manner. After administration of the drugs, the reaction time was measured at 0, 15, 30, 45 & 60 minutes.

Acetic acid –induced writhing method:

This test was performed as described by Gautam *et al.*, IJPSR, 2013. In this method, mice were divided in four groups of six each. The animals were pretreated with drugs 45 minutes before induction of writhing. The animals received the standard drug aspirin (20mg/kg, i.p) which served as reference standard. Analgesic activity of methanolic extract of *Coldenia procumbens* (200 mg/kg & 400 mg/kg, p.o) was assessed by counting the number of writhes induced by 0.6% acetic acid (10 ml/kg, i.p). The number of writhes per animal was counted for the next 20 minutes. Percentage protection against abdominal constriction was taken as an index of analgesia.

It was calculated as:

$$\frac{\text{Number of writhing in control group} - \text{Number of writhing in treated group}}{\text{Number of writhing in control group}} \times 100$$

Statistical Analysis:

The mean value ± SEM was calculated for each parameter. The results were analyzed statistically by ANOVA followed by Dunnett's test. The minimum level of significance was fixed at

p<0.01.The results of experiments by proper statistical analysis are tabulated in following tables respectively.

RESULTS AND DISCUSSIONS [12-14]:

Screening of *in vivo* analgesic activity of crude extract: Hot plate method:

TABLE 1: ASSESSMENT OF ANALGESIC EFFECT OF METHANOLIC EXTRACT OF *C. PROCUMBENS USING* HOT-PLATE METHOD

S.No	Treatment group	Dose(ml/kg)	Basal reaction time (sec)	Reaction time (sec)		
			Paw licking	Paw licking		
				30 min	60 min	120 min
1	Normal saline	10	3.3±0.21	3.6±0.31	2.8±0.20	3.4±0.34
2	Pentazocine	10	3.3±0.33	15.3±1.37**	18.0±.29**	19.0±0.85**
3	CPM-1	200	2.6±0.22	4.6±.26	4.0±0.25	5.6±0.30**
4	CPM-2	400	3.0±0.29	4.0±0.44	4.2±0.45	3.5±0.42

Results expressed as Mean±SEM from six observations; (CPM=*Coldenia procumbens),* ** indicates P < 0.01 as compared control (10 ml/kg of normal saline), One-way ANOVA followed by Dunnett's test.

From the results the Methanolic extract of *C.procumbens* at doses (200 and 400mg/kg, i.p) showed significant (P< 0.01) increase in the mean basal time. The highest nociception inhibition of stimulus exhibited by Methanolic extract of *C.procumbens* (200mg/kg) was observed at 120min. The hot plate method has to be found to be suitable for evaluation of centrally acting analgesics. In centrally acting analgesic method, the drug 200mg/kg dose was found to be significantly effective. The 400mg/kg dose was found to be ineffective in this analgesic model for evaluating centrally acting drugs.

Tail – flick method:

TABLE 2: ASSESSMENT OF ANALGESIC EFFECT OF METHANOLIC EXTRACT OF *C. PROCUMBENS USING* TAIL-FLICK METHOD

S.No	Treatment group	Dose (10ml/kg)	Basal reaction time (sec)	Reaction time (sec)			
				15 min	30 min	45 min	60 min
1	Normal saline	10	3.3±0.21	3.2±0.36	3.1±0.23	3.0±0.21	3.6±0.31
2	Pentazocine	10	3.3±0.33	15.3±1.37**	19.2±0.61**	16.0.±1.0**	16.4±0.90**
3	CPM-1	200	3.8±0.24	5.9±0.62*	5.5±0.34**	5.4±0.52*	5.2±0.48**
4	CPM-2	400	4.0±0.25	5.5±0.34	4.1±0.31	3.8±0.24	4.3±0.37

Results expressed as Mean±SEM from six observations; (CPM=*Coldenia procumbens),* ** indicates P < 0.01& * indicates P < 0.05 as compared control (10 ml/kg of normal saline), One-way ANOVA followed by Dunnett's test.

The extract showed significant analgesic effect at all tested dose levels. In tail flick method, the methanolic extract of *C. procumbens* at a dose of 200 mg/kg showed significant activity. In tail flick test the methanolic extract exhibited a dose-dependent increase in the tail flick latency in the mice. The results were significant (p < 0.01) for 200 mg/kg at 15 min as compared to control.

Tail – immersion method:

TABLE 3: ASSESSMENT OF ANALGESIC EFFECT OF METHANOLIC EXTRACT OF *C. PROCUMBENS USING* TAIL-IMMERSION METHOD

S.No	Treatment group	Dose (10 ml/kg)	Basal reaction time (sec)	Reaction time (sec)			
				15 min	30 min	45 min	60 min
1	Normal saline	10	2.8±0.20	3.2±0.36	3.3±0.21	3.4±0.34	3.6±0.31
2	Pentazocine	10	3.3±0.33**	15.3±1.37**	16.0±1.02**	16.4±0.90**	18.0±1.29**
3	CPM-1	200	2.8±0.24	5.8±0.59*	6.5±0.30**	7.0±0.25**	5.1±0.31**
4	CPM-2	400	3.2±0.39	6.0±0.56*	6.4±0.58*	6.2±0.49*	5.1±0.48*

Results expressed as Mean±SEM from six observations;(CPM=*Coldenia procumbens*), ** indicates P < 0.01& * indicates P < 0.05 as compared control (10 ml/kg of normal saline), One-way ANOVA followed by Dunnett's test.

The methanolic extract of *C.procumbens* at a dose of 200mg/kg showed significant analgesic activity. Tail immersion method has been found to be suitable for evaluation of centrally acting analgesics. In centrally acting analgesic method, the drug 200mg/kg dose was found to be highly significant. The 400mg/kg dose was found to be less effective in this analgesic model for evaluating centrally acting drugs.

Acetic acid – induced writhing test:

TABLE 4: ASSESSMENT OF ANALGESIC EFFECT OF METHANOLIC EXTRACT OF *C. PROCUMBENS ON* ACETIC ACID-INDUCED WRITHING TEST IN MICE

S.No	Treatment group	No. of writhes (per 10 min)	Inhibition (%)
1	Normal saline (10 ml/kg)	59±3.50	-
2	Test drug-I (200 mg/kg)	14.75±2.17	76.7
3	Test drug-II (400 mg/kg)	13.75±2.32	75.O
4	Acetyl salicylic acid (20mg/kg)	23.5±1.55	60.2

The methanolic extract of *C. procumbens* (200mg/kg) significantly reduced the number of abdominal constrictions induced by acetic acid compared to control group. Maximum inhibition of writhing response by methanolic extract of *C. procumbens* (200mg/kg) was 76.7% which was comparable to aspirin response 60.2% at 20 mg/kg. 200mg/kg & 400mg/kg doses were found to be effective in acetic acid- induced abdominal constriction method, which is used to evaluate peripherally acting analgesics. The methanolic extract of *C. procumbens* inhibited the acetic acid induced pain with potency compared to the aspirin. The standard drug aspirin which inhibit the peripheral pain induced by direct action of acetic acid in the abdomen by inhibiting the prosaglandin secretion.

The results showed significant analgesic activity against thermal stimuli. The analgesic studies revealed that the methanolic extract of *Coldenia procumbens* whole plant exhibited potent analgesic (central and peripheral analgesic activity) effect against thermal noxious stimuli.

SUMMARY AND CONCLUSION: The present study is therefore to scientifically investigate the analgesic potential of methanolic extract of whole plant *Coldenia procumbens linn*. *In vivo* Analgesic potential of extract was determined by the Hot-plate method, Tail-flick method, Tail-immersion method and Acetic acid-induced Writhing test carried out on mice. The analgesic effects of methanolic extract of *C. procumbens* in various models of pain were found to be analogous. The stimulus may be thermal (hot plate, tail flick and tail immersion tests) or chemical (writhing test). The hot plate method, tail flick and tail immersion methods have been found to be suitable for evaluation of centrally acting analgesics. The nociceptors seem to be sensitized by sensory nerves. The involvement of endogenous substances such as prostaglandins may be minimized in these models. In centrally acting analgesic methods, the drug 200 mg/kg dose was found to be significantly effective. Acetic acid-induced abdominal constriction is a sensitive method for screening peripheral analgesic effect of compounds. This may be due to increase in concentration of PGE2 and PGF2α in the peritoneal fluid. *Coldenia procumbens* methanolic extract at doses (200mg/kg & 400mg/kg) were found to be effective in acetic acid-induced abdominal constriction method that is used to evaluate peripheral analgesics. *Coldenia procumbens* methanolic extract at a dose 200 mg/kg showed the most significant *in-vivo* central analgesic activity[11]. Both the 200 mg/kg and 400 mg/kg doses were significant ($P<0.01$) as comparable to the standard.

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