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Analgesic activity of *Cryptostegia grandiflora* (Roxb.)R.br. leaves methanol extract using mice

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ABSTRACT

Objective: To evaluate analgesic property of leaf methanol extract of *Cryptostegia grandiflora* (Roxb.) R.Br. using mice. **Methods:** Analgesic activity was evaluated by abdominal writhing and tail flick methods using Swiss albino mice. Acetyl salicylic acid was used as standard drug. **Result:** The leaf methanol extract did not show any signs of toxicity upto 5000 mg/kg b.w. The leaf methanol extract (LME) was tested at three different dosages viz., 250, 500 and 750 mg/kg b.w. orally. All the three doses of LME showed significant ($P<0.001$) analgesic activity, among them dose at 750 mg/kg b.w. showed 62.93% inhibition, but less effective than standard acetyl salicylic acid (93.70%) as revealed by writhing test. In Tail-flick model, the LME at the dose of 500 mg/kg b.w. showed significant activity ($P<0.001$) when compared to the other two doses as evidence by the increase in the latency of tail response to thermal stimulation. **Conclusions:** This investigation revealed that the *C. grandiflora* LME demonstrated significant analgesic effect in both abdominal writhing and tail flick models. Among the three different doses tested, the 500 mg/kg b.w. was found to be more potent. The results of this investigation revealed that the LME of *C. grandiflora* possesses significant analgesic property and supported the traditional medicinal claims on C. grandiflora.

1. Introduction

Pain is an unpleasant sensory and emotional experience [1] occurred by blocking pain signals interfering with the brain signals. Pain motivates the individual to withdraw from damaging situations, to protect a damaged body part while it heals, and to avoid similar experiences in the future [2] if not, leads to many diseases such as tumor, physical trauma, surgical procedures, noxious chemical stimulation etc [3]. Most of the pain relieving drugs produced pronounced side effects on the physiology of the body such as sweating, apprehension, nausea and palpitation [4]. Hence there is an utmost need for an alternative effective treatment against pain without side effects. Natural occurring bioactive compounds in the plants are believed to be an important source with potential therapy for pain. Many drugs are used to relieve the pain. Morphine [5] and aspirin [6] are used pain killer since time immemorial. Any pain caused primarily by stimulation of the nociceptor can be said to be nociceptive pain [7]. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems [8].

Several plants are reported to possess analgesic property as many investigators screened the plant extracts and their phytoconstituents for their analgesic property viz., *Bowdichia virgilioides* [9], *Capparis ovate* [10], *Urtica circularis* [11], *Phlomis umbrosa* [12], *Delonix elata* [13].

Cryptostegia grandiflora (Roxb.) R. Br. (Family: Asclepiadaceae) is widely distributed throughout tropical Africa, Madagascar and some parts of India [14, 15]. The juice of aerial parts of *C. grandiflora* are reported to, produce caoutchoue when exposed to sunshine [16]. It is also reported that this plant decoction is consumed to treat nervous disorders [17]. This plant species is also reported to possesses various biological activities like antioxidant [18], antitumour [19] antiviral [20] and control the schistosomiasis [21]. The aqueous solution of ethanol extract of aerial parts exerted significant hypoglycemic action in normal rabbits

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[22, 23] and the latex derived from this plant have proteolytic, bacteriolytic activity and possess relevant enzymatic activities against pathogenic related proteins [24, 25]. Rigorous literature survey revealed that there are no reports available regarding analgesic property of *C. grandiflora*, hence the present study was undertaken to evaluate the analgesic activity of the leaf methanol extract (LME) of *C. grandiflora* to substantiate its traditional claims as decoction to treat nervous disorders through scientific evaluations.

2. Materials and methods

2.1. Plant material and extraction

The leaves of *C. grandiflora* were collected in December 2009 from the villages near by Davanagere district, Karnataka, India. The plant material was identified and authenticated by Prof.Y.L. Krishnamurthy and plant herbarium has been deposited (No: KU/SD/SP229) in the, Department of Botany, Kuvempu University.

The leaves were cleaned with deionized water and were shade dried, grounded porously by using mechanical blender and passes through 40-mesh sieve. About 1 kg of powdered material was loaded into four Soxhlet timbles of 250 g each and extracted using methanol for about 48 h. The extracts were filtered (Whatman No.1 filter paper) and concentrated in vacuum under reduced pressure using rotary flash evaporator (Buchi, Flawil, Switzerland) and then the extract was kept on water bath for complete evaporation of solvent. Finally the dried extract was preserved in air tight container until use.

2.2. Animals

Healthy Swiss albino mice weighing 20-25 g were procured from Central Animal House, National College of Pharmacy, Shimoga, Karnataka, India and were housed at 23±2 ℃, humidity 55-60% and were fed with standard commercial pellet diet (Durga Feeds and Foods, Bangalore) and water ad libitum. All the animals were acclimatized for one week before the experiments, and all experiments were carried out according to the institutional animal ethics committee guidelines (Re: NCP/IAEC/CL/07/12/2010-11).

2.3. Acute toxicity study

Acute oral toxicity [26] study was performed as per OECD-423 guidelines (acute oral toxic class method). Albino mice (n = 6) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for overnight providing only water, after which the methanol extract was administered orally at the dose level of 50 mg/kg body weight by intragastric tube and observed for 72 hrs. This dosage was gradually increased up to 5000 mg/kg until any behavioral changes or mortality was observed.

2.4. Preparation of extract

The three different concentrations of LME and the standard drug were prepared for oral administration in the form of suspension in 1% DMSO as suspending agent.

2.5. Analgesic activity

2.5.1. Abdominal writhing method

The abdominal writhing method described by [27] was carried out to measure the analgesic action. Analgesic activity of the crude LME was carried out using adult Swiss albino mice of either sex weighing 20-25 g, five groups with 6 animals per group were selected for abdominal writhing method [28]. Group I animals were treated with 0.6% acetic acid (dose 10 ml/kg) intraperitoneally. After 5 min of acetic acid administration, numbers of writhes were counted for 20 min. This reading was taken as control. Group II, III and IV were administered orally with the 1% DMSO dissolved LME at the dose of 250, 500 and 750 mg/kg body weight respectively. Group V was administered with standard drug acetyl salicylic acid (100 mg/kg b.w.) and was used for the comparison of analgesic activity. After one hour incubation all the groups except group I animals were administered with acetic acid. After 5 min, each group mice were observed for the number of writhes for the duration of 20 min. The mean value for each group was calculated. A reduction in the writhing number compared to the control group was considered as evidence of analgesia. The percentage inhibition of writhing was calculated as: % Inhibition = A-B / A X 100. Where, A = mean number of writhes produced by the control group and B = mean number of writhes produced by the test groups.

2.5.2. Tail flick method

Analgesic effect of the LME was determined by the tail-flick method as described by Sewell and Spencer (1976) [29]. Swiss albino mice of either sex weighing between 20-25 g were divided into 4 groups of six mice in each group. Group I mice were treated with normal saline (10 m1/kg b.w.). Group II, III and IV were administered orally with the 1% DMSO dissolved LME at the dose of 250, 500 and 750 mg/kg b.w. respectively. One to two centimeter of the experimental mice tail was immersed in warm water kept constant at 50 ℃. The pain reaction time was the time taken by the mice to deflect their tails. The first reading is discarded and the reaction time was taken as a mean of the next two readings. The latent period of the tail-flick response was taken as the index of analgesic activity and was determined before and at 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 h after the administration of drugs. The maximum reaction time was fixed at 0.5 h (30 min). The maximum possible analgesia (MPA) was calculated according to the method of Idid et al., (1992) [30].

2.6. Statistical analysis

The data of analgesic activity was expressed as mean ± S.E.M of six animals in each group. The statistical analysis was carried out using one way ANOVA followed by Tukey's t-test. The difference in values at $P \leqslant 0.01$ was considered as statistically significant.

3. Results

3.1. Acute toxicity study

Acute toxicity (LD_{50}, p. o.) of leaf methanol extract of *C. grandiflora* (LD_{50}) for oral administration was evaluated using mice. It is inferred that up to 50 to 5000 mg/kg b. w. did not show any observable behavioral changes or mortality. It is found to be a safer dose for administration and the LD_{50} was taken at 1/10th of the examined concentration of the extract.

3.2. Analgesic activity

3.2.1. Writhing method

The number of abdominal writhes observed during 20 min. period after 0.6% acetic acid administration through i.p. in control group was 85.80 ± 0.86. The treatment of methanol extract at the dose of 250, 500 and 750 mg/kg b.w. reduced the number of writhes to 36.80 ± 0.58 (57.10 % analgesia), 36.80 ± 0.58 (75.05% analgesia) and 31.80 ± 0.86 (62.93 % analgesia) respectively. But the effect of LME was slightly less potent than the standard drug acetyl salicylic acid with 05.40 ± 0.51 writhes, eliciting 93.70% analgesia, Although the effect was found to be significant (P<0.01) and the data is depicted in the Table 1.

3.2.2. Tail flick method

During the tail flick experiment, the effect of LME in mice was observed over a period of 3h, while 1% DMSO administered to animals as control group remained non-toxic on the latent period of tail-flick response. Before the administration of LME, the initial reading was documented. Following oral administration at three different concentrations (250, 500 and 750 mg/kg b.w.) of LME using gavage, the effect of LME was significant (P<0.01) over 3h observation period. Analgesic effect after the LME administration was recorded at every 30 min. time intervals and was found to be evident within 0.5h of experiment. At 250 mg/kg dosage, the analgesia was observed to be increased from 4.40 ± 0.24 to 9.20 ± 0.37%. Similarly, at 500 mg/kg dosage, the analgesia increased to 10.80 ± 0.37%. While at 750 mg/kg b.w. dosage the calculated analgesia (MPA) value was (P< 0.01) significantly decreased to 9.60 ± 0.68% against the control group. The effect of LME on analgesic response induced by noxious heat (50 ℃) as shown in Table 2.

4. Discussion

Medicinal plants formulations are widely used in several therapies including pain killing and nervous disorders. Leaves of medicinal plants are common substance of many folk and traditional herbal medicines. *C. grandiflora* is a toxic vine, and though it is a toxic plant, the leaf decoction of this plant is consumed to treat various nervous disorders and wound healing [31].

Acute toxicity study of LME of *C. grandiflora* revealed that up to 5000 mg/kg b. w. did not show any observable changes in the behavior or mortality of animals. Hence, one tenth of this dose was considered to be the safer dose for administration.

The preliminary phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, steroids, saponins, tannin and phenolic compounds. Many investigators have reported the actions of secondary metabolites such as flavonoids and alkaloids played a major role in analgesic activity [32, 33]. The presence of secondary metabolites like saponins, flavonoids, tannins, and terpenoides may be attributed for analgesic activity [34]. However, alkaloids are well known for their ability to inhibit pain perception [35, 36], whereas flavonoids are primarily targeting prostaglandin synthesis involved in pain perception, indicating that flavonoid components of the plant extract might be responsible for analgesic property of the extract [37,38,39].

In the present study, analgesic activity of LME of *C.*

Table 1

Analgesic activity of *C. grandiflora* leaf methanol extract by writhing method.

Drug treatment	Dose	Number of writhes	% inhibition of writhings
Control(acetic acid)	10 ml/kg (i.p.)	85.80± 0.86	–
Acetyl salicylic acid	100 mg/kg (p.o.)	05.40± 0.51	93.70**
Leaf methanol extract	250 mg/kg (p.o.)	36.80± 0.58	57.10**
	500 mg/kg (p.o.)	21.40± 1.03	75.05**
	750 mg/kg (p.o.)	31.80± 0.86	62.93**

Values are the mean ±S.E.M. of six mice. Symbols represent statistical significance.*P < 0.05, ** P < 0.01, ns – not significant, as compared to control group.

Table 2

Analgesic activity of *C. grandiflora* leaf methanol extract by tail flick method

Group(N)	Dose (p.o.)	0.5h	1.0h	1.5h	2.0h	2.5h	3.0h
Contro(1% DMSO)	10 ml/kg	3.03±0.05**	2.8 ± 0.06**	2.9 ± 0.04**	3.2±0.08**	3.1±0.02**	3.4±0.05**
Leaf Methanol extract	250mg/kg	9.20±0.37**	7.20±0.37**	7.0 ±0.45**	5.60±0.40**	5.80±0.20**	4.40±0.24**
	500 mg/kg	10.80±0.37**	9.60±0.24**	9.20±0.20**	8.80±0.49**	9.20±0.37**	8.20±0.20**
	750 mg/kg	9.60±0.68**	9.60±0.40**	8.60±0.40**	8.40±0.57**	8.20±0.58**	7.60±0.40**

Values are the mean ±S.E.M. of six mice. Symbols represent statistical significance.*P < 0.05, ** P< 0.01, ns – not significant, as compared to control group.

grandiflora was evaluated by using acetic acid induced abdominal writhing and tail flick method in mice. In acetic acid induced experiment, animal models react with unique abdominal stretching behavior which is called writhing. The reduction in abdominal writhing indicates the percentage levels of analgesia in the acetic acid writhing reflex model [40] in which the pain is due to the release of free arachidonic acid from phospholipid tissue [41] via, cyclooxygenase (COX) and prostaglandin biosynthesis [42]. The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics and the response is thought to be mediated by peritoneal mast cells [43], acid sensing ion channels [44] and the prostaglandin pathways [45]. Prostaglandins E2 and F2 α are reported to be increased in the peritoneal fluid of mice due to administration of acetic acid, this could be produced by neutrophil polynuclear cells but also by destruction of macrophages [46, 47]. The significant pain reduction of LME might be due to the presence of alkaloids and flavonoids analgesic principles acting against the prostaglandin pathways.

The centrally acting analgesics generally raise the pain threshold of mice towards heat [48]. The thermal induced nociceptive tests are more sensitive to opioid receptors and non-thermal tests are sensitive to κ-opioid receptors as they are G-protein-coupled receptors (GPCRs) [49, 50, 51]. The narcotic analgesics inhibit both peripheral and central mechanism of pain, while nonsteroidal anti-inflammatory / analgesics agents (NSAIAs) inhibit only peripheral pain [52, 53]. The inhibition of pain could take place not only from the presence of opioids and/or opiodiomimetics but also from bio-active compounds and secondary metabolites like phenolic and steroidal constituents [54, 55]. Significant analgesic response was observed at the dose of 250 and 500 mg/kg b.w. However, steady state response was not observed to be maintained at higher concentration of 750 mg/kg b.w. Among the three doses, 500 mg/kg b.w. showed significant ($P< 0.01$) analgesic response against acetic acid induced writhing. The significant effect of these extracts is due to the presence of a biological active constituent in higher levels or due to the effect of more than one phytoconstituents. The LME of *C. grandiflora* at three different doses showed good peripheral analgesic activity by decreasing the number of writhes and exhibited central analgesic activity by showing significant ($P<0.001$) effect on the latent period of tail-flick response at the dose of 500 mg/kg b.w. The present study revealed that the leaf methanol extract of *C. grandiflora* exhibited significant analgesic property but less effective than the standard reference.

Conflict of interest statement

We declare that we have no conflict of interest.

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