Research Article

Analgesic and anti-nociceptive activity of hydroethanolic extract of *Capparis decidua* (Forssk.) Edgew.

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Abstract

Objectives: To study the analgesic and anti-nociceptive activity of hydro-ethanolic stem extract of *Capparis decidua* (Forssk.) Edgew. **Materials and methods**: Wistar rats and Swiss albino mice were selected for study of analgesic and anti-nociceptive activity of *Capparis decidua* hydro-ethanolic extract (CDHE) at doses 50, 100 and 200 mg/kg p.o. a variety of models viz. acetic acid induced writhing model (female mice), Eddy's hot plate (mice) and tail flick model for analgesic study, and formalin-induced paw licking model (mice) were usedforanti-nociceptivestudy. **Results**: In acetic acid induced writhing model and in the hot plate model method used with indomethacin standard drug, the maximum effect was observed at 60 min at a dose of 200 mg/kg p.o., which was higher than the standard drug morphine sulfate (1.5 mg/kg i.p.) while in the tail flick model, effect was comparable with morphine sulfate. In formalin-induced paw licking model, administration of CDHE completely abolished the early phase at 100 and 200 mg/kg p.o. and in the late phase, the result of CDHE (200 mg/kg p.o.) was superior than indomethacin (10 mg/kg p.o.). CDHE was effective in both non-narcotic and narcotic models of nociception, signifying its possible action via peripheral and central mechanism. It also abolished the early phase in formalin-induced paw licking model, signifying complete inactivation of C-fiber at higher dose. **Conclusion**: The activity can be recognized to the phyto-constituents viz tannins, diterpenes, triterpenes and steroids present in the CDHE extract. In conclusion, CDHE can be developed as a potent analgesic and anti-nociceptive agent in future.

***Keywords*:** Analgesic, acetic acid, *Capparis decidua* , Eddy′s hot plate, hydro-ethanol extract, tail flick

Introduction

The use of herbal plant products is growing in many segments of the population. According to an estimate 80-90% of the world's population leading herbal plants for their medicine. The majority of the synthetic drugs used at present for analgesic and antinociceptive effect with many side and toxic effects. Herbal plants still stand for a large untapped resource of structurally novel active compounds that might serve as lead for the growth of novel drugs (Ahmad et al., 1992). Numerous medicines of herbal plant origin with analgesic and anti-nociceptive activity had been used since long time without any adverse effect. In north East India is regard as as one of the "hotspots" for biodiversity in India, since out of the 1500-1600 species of medicinal herbal plants available in India, almost 350 species belong to Assam, and many of these traditionally used plants have not yet been investigated scientifically. The Greek name kapparis is from the Persian kabar, "caper". Pickled capers have been used as a condiment for over many years (Bown, 2008). The plant is commonly well-known as Caper berry in English, Karira in Sanskrit, Karer or Kurrel in Hindi, Kerada in Gujarati, Nispatige in Kannad, Nepati in Marathi, Kair in Punjabi, Shengan in Tamil and Enugadanta in Telegu, Titali in Urdu, belong to the family Capparidaceae. Herbal plant naturally grows in dry, exposed habitat, often on foothills, in wastelands. It is originate mostly in the deserts, Punjab and Sind, especially of Rajputana etc, (Khare, 2007) southwards to Karnataka and Tamil Nadu, (Gupta, 2008) growing wild in Western Ghats, Rajasthan and Gujarat (Kirtikar and Basu, 2008). Caper buds are both wild-collected and cultivated; plants

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grown in cultivation tend to be spineless. It have cultivated in well drained, sandy soil in sun. It is propagate by seed sown in autumn or spring by ripe woodcutting in summer at 19-24^{0}C (Bown, 2008). It has been revealed in ayurveda that the bark has an acrid, sharp, hot taste; analgesic, diaphoretic, laxative, in dropsy ground, (Anonymous, 2007) anthelmentic, asthma, ulcers and boils, vomiting, piles and all inflammations diseases. The root of *Capparis decidua* bark is pungent and is given in cases of intermittent fevers and rheumatism (Gupta, 2010; Chopra et al., 2006). It is applied externally to ribs in case of pleurisy (Gupta, 2010). Phytochemical screening of *Capparis decidua* discovered high contents of isothiocyanate glucoside, glucocapparin, stachydrine, n-triacontane β-carotene and β-sitosterol. It has been reported the presence of n-triacontanol, n-pentacosane and phthalic acid. The *Capparis decidua* flowers having antimicrobial volatile sulphur compound (0.4%) (Anonymous, 2007). A variety of preparations of *Capparis decidua* are available like powder of Leaves & root (Khare, 2007) 50-125 mg, juice of plant, and infusion of root-bark (1 in 10), dose: ½ - 1 ounce, (Nadkarni, 2009) used in treatment of diseases.

Materials and methods

Collection of plant material

Clean tender stem of *Capparis decidua*, approximately 1 kg collected during feb 2011, were used. The herbal plant was authenticated by Dr. D.M. Pandey Professor of plant taxonomist and scientist of Botanical survey of India, Jodhpur Rajasthan India, at 23 March 2011. The herbal plant material was well air-dried at room temperature. The dried stem were ground to fine powder and stored in air tight container.

Preparation of extract of *Capparis decidua*

Fresh stem of *Capparis decidua* were cleaned and dried under shade, then after grinded and stored in an air-tight container. They were (250 g) soaked in 1000 ml of distilled water-ethanol (50:50) for 75 h in separate beakers. It was stirred every 19 h with a sterile glass rod. The solvent was filtered every third day by muslin cloth or Whatman's filter paper No 1. The filtrate obtained was concentrated by rotary vacuum evaporator (Equitron, Roteva) at 50-60° C. The *Capparis decidua* hydroethanolic extract (CDHE), consequently obtained, and was transferred to a Petri dish and kept over water bath (50˚ C) completely evaporated. It was stored at 4˚ C for future use. Recovery was 18.07% (w/w).

Phytochemical screening

Freshly prepared CDHE was subjected to phytochemical screening tests for various phyto-constituents by standard methods (Harbome, 1991).

Animals and treatment regimes

The analgesic and anti-nociceptive study were performed on Swiss albino mice (20-30 g) and Wistar rats (120-130 g). They were fasted during the night before the experiment and allowed free access to drinking water. All the studies were conducted with the guidelines of care and use of laboratory animals, and promulgated through the Institutional Animal Care Committee, CPCSEA, India (Reg. No. CPCSEA). *Capparis decidua* hydro-ethanolic extract (CDHE) dose was administered orally like 100, 200, 400, 800, 1000 and 2000 mg/kg to the group of mice (n=3) and the percentage mortality was recorded for a time of 24 h. all through the first 1 h after the drug administration, the mice were experimental for any unpleasant behavioral change and the parameters observed were like hyperactivity, grooming, convulsions, loss of righting reflex, sedation and salivation, respiration, urination and defecation (Chopra et al., 2006). Based on the toxicity study, direct limit test was determined. Initially a particular dose, on the basis of the above study, *Capparis decidua* hydro-ethanolic extract (CDHE) dose was administered to single female rat and the rat was observed for 40-48 h with close up surveillance to initial 3-4 h (same as in case of first rat) after 48 h (of the second administration), same dose was administered in two or more female rats and the observation was done same as for the previous rat. The rats were supervised for 14 days and no adverse observation was found. The weight of the animals was recorded on 7th to 14th day. Animals were separated into five groups, and each group having six animals. The first group (Group I) served as a control group. The second (Group II) was used as the standard. Three groups (Group III, IV and V) received CDHE at three different doses like (50, 100 and 200 mg/kg p.o.). The research was conducted in accordance with the Animal Care Committee, CPCSEA India.

Reagents

Indomethacin (CDH Pvt Ltd) and morphine sulfate (Drugs India Pvt. Ltd. Jodhpur, Rajasthan) were used for the study. Distilled water was used as vehicle and all the chemicals and solvents were of analytical grade.

Analgesic and anti-nociceptive activity

Acetic acid induced writhing model in mice

The animals were pretreated with standard drugs, 30 min earlier than induction of writhing. The Group I animals received vehicle (control) and Group II animals received the standard drug indomethacin (10 mg/kg p.o.). Analgesic activity of *Capparis decidua* hydro-ethanolic extract CDHE doses like 50, 100 and 200 mg/kg p.o. (Group –III, IV and V) was assessed by together with the number of

writhes induced by 0.7% acetic acid. The number of writhes per animal were noted for 20 min (Vogel, 2002). Percent decrease in writhing syndrome was calculated and compared with the standard drug. Percent decrease indicates the percentage protection against abdominal constriction which was taken as an index of analgesia.

It was calculated as: {(Wc –Wt) × 100}/Wc

Where, Wc = number of writhing of the control group Wt = number of writhing of the treated group

Eddy's hot plate model in mice

The animals established vehicle (Group I) and standard drug indomethacin (10 mg/kg p.o.) (Group II). Analgesic activity of at the *Capparis decidua* hydro-ethanolic extract CDHE at doses like 50, 100 and 200 mg/kg p.o. (Group III, IV and V) was assessed by counting the reaction time of treated groups. The reaction time was determined at 0, 30, 60, 90 and 120 min (Witkin et al., 1961). A cut-off time of 20 sec was considered.

Tail flick model in rat

The animals established vehicle (Group I) and standard drug (Indomethacin 10 mg/kg p.o.) (Group II). Analgesic activity of *Capparis decidua* hydro-ethanolic extract CDHE at doses like 50, 100 and 200 mg/kg p.o. (Group III, IV and V) was assessed by observing the reaction time of the treated groups (Eddy and Leimbach, 1953). Following the administration of drugs, the reaction time was determined at 0, 30, 60, 90 and 120 min.

Formalin- induced paw licking model in mice

In this model method, the animals established vehicle (Group-I) and standard drug (Indomethacin 10 mg/kg p.o.) (Group II). Anti-nociceptive activity of CDHE at drug doses like 50, 100 and 200 mg/kg, p.o. (Group III, IV and V) was assessed by observing the reaction time in the treated groups. 15 minutes after treatment, 20 µml of 1% formalin was injected subcutaneously route in dorsal surface of the hind paw and the time spent for licking the paw injected with formalin was counted for 30 min after formalin injection and considered as indicative of the pain stimuli (Abbott et al., 1995; Turner, 1965). The formalin test has two distinctive phases possibly reflecting diverse types of pain. The first phase peaked at 5 min and the second phase at 20–30 min after formalin injection. This represent, neurogenic and inflammatory responses, respectively.

Statistical analysis

The statistical analysis of experimental data was done with one-way analysis of variance, and SPSS software (version 11.5). $P< 0.01$ was considered as highly significant.

Results and discussion

Phytochemical screening

The phytochemical screeningor test of CDHE stem showed the presence of constituents like diterpenes, tannins by ferric chloride and gelatin test triterpenes by Salkowski's and Liberman Buchardt's test and steroids by Salkowski's and Liberman Buchardt's test.

Acute toxicity studies

Oral administration of CDHE up to 2 g/kg did not generate any side effects in the normal behavior of the mice.

Analgesic and anti-nociceptive activity

Any damage of tissue is linked with pain and inflammation. Analgesics drugs can act on peripheral or central nervous system. Peripherally acting analgesics drugs act by inhibiting the generation of impulses at chemo-receptors site of pain, whereas centrally acting analgesics not only raise the threshold for pain and also alter the physiological response to pain and repress the patient's anxiety and apprehension. Pain and inflammation are an essential prelude to the repair process (Hunskaar and Hole, 1997). Acetic acid produces inflammatory pain by inducing capillary permeability and liberating endogenous substances that stimulate pain nerve ending. Acetic acid is also known to increase PGE1 and PGE2 peripherally (Shreedhara et al., 2009). NSAIDs can inhibit COX in peripheral tissues and as a result interfere with the mechanism of transduction of primary afferent nociceptors (Kumar et al., 2001). The role of analgesic activity of CDHE might be probably due to the obstruction of the effect or the release of endogenous substances that excite pain nerve endings similar to that of indomethacin and NSAIDs. Consequently the decrease in the number of writhing indicates that CDHE might put forth anti-nociceptive activity by embarrassment of prostaglandin synthesis or action of prostaglandin hormone. In the hot plate model method, nociceptive reaction toward thermal stimuli in mice is a well-validated model for recognition of opiate analgesics as well as numerous types of analgesics drugs from spinal origin (Adzu et al., 2003). The tail flick model method is also used to evaluate analgesic agents acting through central nervous system. CDHE the formalin-induced paw licking model method comprises of early phase and late phase. The near the beginning phase (immediately after injection) seems to be produce by C-fiber activation due to the peripheral stimulus. The late phase (initial approximately 20 min after formalin injection) appear to depend on the combination of an inflammatory reaction, activation of NMDA and non-NMDA receptors and NO cascade (Alhaider et al., 1991) in the peripheral tissue and the functional changes in the dorsal horn of the spinal cord (Davidson and Carlton, 1998). In our study, CDHE completely abolishes the before time phase at

the dose 100 and 200 mg/kg p.o. signifying complete inactivation of C- fiber in the early phase. DCHE decreased the reaction time in dose dependent manner in the late phase also which might recommend that CDHE causes partial inactivation of NMDA and non-NMDA receptors. Our studies have shown analgesic activity in the hydro-ethanol extract (Anonymous. 2007). Analgesic activity of hydro-ethanol extract of *Capparis decidua* in the present study showed superior effect in both narcotic and non narcotic models, while in the hydro-ethanol extract, the activity was more important in non narcotic models (acetic acid induced writhing) only. The hydro-ethanol extract haveing flavonoids, steroids, triterpenes, diterpenes, alkaloids and tannins but the hydro-ethanol extract contains steroids, triterpenes, diterpenes and tannins. Hence, significant analgesic activity in hydro-ethanolic extract might be recognized to the presence of these bioactive principles.

Table 1. Analgesic activity of CDHE in female albino mice in acetic acid induced writhing method

Groups	Treatments	Dose mg/kg	No. of writhing	Percentage reduction %
1	Control	-	77± 1.67	0.00
2	Indomethacin	10	8.33± 0.63	86.45
3	Plant extract	50	11.8± 0.79	80.76
4	Plant extract	100	6.83± 0.83	88.89
5	Plant extract	200	4.83± 0.47	92.14

Values are expressed in terms of mean ± SEM, n=6 in each group, P≤ 0.01 statically highly significant as compared with control with control group.

Table 2. Analgesic activity of CDHE in Eddy's hot plate model in mice

Groups	Treatments	Dose mg/kg	Reaction time in minutes				
			0 min	30 min	60 min	90 min	120 min
1	Control	-	4.99±0.25	5.03±0.09	5.70±0.15	5.31±0.35	4.69±0.20
2	Morphine	1.5	8.55±0.25	10.1±0.09	12.3±0.15	13.8±0.35	14.9±0.20
3	Plant extract	50	8.80±0.89	11.7±0.65	13.7±0.63	14.4±0.85	16.5±0.88
4	Plant extract	100	12.0±0.66	14.4±0.38	15.9±0.33	17.6±0.74	15.4±0.45
5	Plant extract	200	13.6±0.52	15.6±0.81	18.2±0.64	15.2±0.48	14.6±0.53

Values are expressed in terms of mean ± SEM, n=6 in each group, P≤ 0.01 statically highly significant as compared with control with control group.

Table 3. Analgesic activity of CDHE in Wistar rat by tail flick method

Groups	Treatments	Dose mg/kg	Reaction time in minutes				
			0 min	30 min	60 min	90 min	120 min
1	Control	-	5.22±0.16	5.56±0.22	5.88±0.17	5.71±0.22	5.81±0.20
2	Morphine	1.5	3.31±0.22	6.10±0.23	16.2±0.71	17.9±0.48	19.9±0.34
3	Plant extract	50	6.17±0.03	6.32±0.23	6.46±0.01	6.56±0.01	6.78±0.01
4	Plant extract	100	7.07±0.02	7.26±0.01	7.37±0.01	7.84±0.01	7.55±0.01
5	Plant extract	200	8.18±0.01	8.34±0.01	8.94±0.01	8.55±0.01	8.44±0.01

Values are expressed in terms of mean ± SEM, n=6 in each group, P≤ 0.01 statically highly significant as compared with control with control group.

Table 4. Anti-nociceptive activity of CDHE in albino mice in formalin induced paw-licking test

Groups	Treatments	Dose mg/kg	Early phase duration of paw licking in seconds	Late phase duration of paw licking in seconds
1	Control	-	59.4± 1.02	143± 1.14
2	Indomethacin	10	23.9± 0.44	36.53± 2.62
3	Plant extract	50	21.4± 0.01	52.81± 1.22
4	Plant extract	100	0.00	36.76± 1.57
5	Plant extract	200	0.00	26.73± 1.11

Values are expressed in terms of mean ± SEM, n=6 in each group, P≤ 0.01 statically highly significant as compared with control with control group

Conclusions

In conclusion, it can be interpreted that CDHE possesses shows potential analgesic and anti-nociceptive properties, which are probably peripherally mediated through inhibition of prostaglandin synthesis as well as central inhibitory mechanism and may be of potential benefit for management of pain. Additional studies on isolation and fractionation of the active components from the leaf of *Capparis decidua* and study on its mechanism of action to ascertain their analgesic and anti-nociceptive properties will throw light on mode of action.

Conflict of interests

The authors pronounce that they have no conflict of interests.

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