

Anti-nociceptive Effect of *Caralluma edulis* on Peripheral and Central Pain Pathways

(Kesan Anti-nosiseptik *Caralluma edulis* terhadap Sistem Sakit Berpusat dan Periferal)

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ABSTRACT

The purpose of the present research was to evaluate the phytochemical content and analgesic effect of *Caralluma edulis* (Ce.Cr). Established methods were used for phytochemical analysis of plant. The anti-nociceptive activity of Ce.Cr was scrutinized using acetic acid-induced writhings, tail immersion and hot plate methods. Ce.Cr was tested positive for the presence of therapeutically active metabolites such as alkaloids, flavonoids, glycosides, phenol, tannins, terpenoids and saponins. Ce.Cr at the dose of 10, 30 and 100 mg/kg inhibited acetic acid-induced abdominal writhes and increase the latency time to thermal stimuli in both tail immersion and hot plate tests, similar to standard drug. These results showed that the ethanolic extract of *Caralluma edulis* possesses anti-nociceptive property.

Keywords: Anti-nociceptive; *Asclepiadaceae*; *Caralluma edulis*

ABSTRAK

Penyelidikan ini bertujuan untuk menilai kandungan fitokimia dan kesan analgesik *Caralluma edulis* (Ce.Cr). Kaedah sedia ada digunakan untuk menganalisis fitokimia tumbuhan. Aktiviti anti-nosiseptik Ce.Cr diteliti dengan menggunakan kaedah asid asetik bergelut, rendaman hujung dan plat panas. Ce.Cr diuji positif untuk kehadiran metabolit terapeutik aktif seperti alkaloid, flavonoid, glikosida, fenol, tanin, terpenoid dan saponin. Ce.Cr pada dos 10, 30 dan 100 mg/kg merencat abdomen bergelut teraruh asid asetik dan meningkatkan masa kependaman untuk rangsangan terma dalam ujian rendaman hujung dan plat panas seperti piawai dadah. Keputusan ini menunjukkan bahawa ekstrak etanol *Caralluma edulis* memiliki sifat anti-nosiseptif.

Kata kunci: *Caralluma edulis*; *Asclepiadaceae*; Anti-nosiseptif

INTRODUCTION

Pain is an awful sensory or emotional experience caused by noxious stimuli and associated with tissue damage. It is a complex multidimensional phenomenon linked to sensory, emotional and cognitive aspects. Pain is an alarming symptom that is protective in nature but accompanied by discomfort. Analgesic drugs relieve pain by acting centrally or peripherally without affecting its cause. For the development of new analgesic agents, there is a need to explore more natural products derived from medicinal plants that have similar analgesic activity and with fewer side effects (De Sousa 2011; Raquibul Hasan et al. 2009).

Caralluma edulis (Edgew.) Bth. Hk. 15-45 cm long succulent herb belongs to family Apocynaceae, commonly known as chunga in Pakistan (Ahmad et al. 2009). Geographically distributed in the Pakistan and Iran. It is used traditionally in the treatment of Alzheimer, diabetes, fever, gastric problems, hypertension, rheumatism, leprosy and parasitic diseases (Adnan et al. 2014). *Caralluma edulis* is reported to possess anti-diabetic (Wadood et al. 1989) and anti-oxidant potential (Ansari et al. 2005). In present investigation the extract of *Caralluma edulis* was screened for anti-nociceptive effect, to validate the traditional use in rheumatism.

MATERIALS AND METHODS

PLANT MATERIAL AND EXTRACTION

Caralluma edulis (10 kg) was purchased in June 2015 from the local market in Islamabad, Pakistan. The collected plant was identified by a taxonomist (Dr. Mushtaq Ahmad). The voucher specimen (422) was deposited to herbarium at Department of Plant sciences, Quaid-a-Azam University, Islamabad. The plant material was dried under shade and powdered. The crushed material was macerated with 70% aqueous-ethanol for the period of 7 days at room temperature, stirring continuously at regular intervals. The extract was filtered via a Whatmann filter paper grade 1. The filtrate was concentrated using rotary evaporator to obtained thick semi-solid paste (Shahnaz et al. 2016).

CHEMICALS

Acetic acid was obtained from Daejung Chemicals & Metals Co., Ltd. Korea. Diclofenac sodium was obtained from Olive Laboratories National Industrial Zone Rawat, Islamabad. Ethanol was obtained from BDH Laboratory Supplies, poole, England and tramadol obtained from Searle Company, Karachi, respectively.

ANIMALS

The experiment was conducted on Balb-C mice of either sex and divided into different groups containing five mice each weighing 25-35 g. Animals were issued from the Department of Pharmacology, Riphah Institute of Pharmaceutical Sciences, Islamabad. They were housed at 23-25°C with 12 h light and dark cycle and received standard food as per protocol and free access to water *ad libitum*. All the Experiments were performed according to the policy of Institute of Laboratory Animal Resources, Commission on Life Sciences University, National Research Council (1996) and approved by Ethical Committee of Riphah Institute of Pharmaceutical Sciences (Ref. No: REC/RIPS/2015/005).

PHYTOCHEMICAL ANALYSIS

Different tests were performed for the detection of various phytochemical classes, according to standard procedure (Evans 1996; Haq et al. 2016; Harborne 1998), with some modifications. Dragendorff's and Mayer's reagents were used for the detected of alkaloids. For the detection of flavonoids, plant filtrate (3 mL) was mixed with potassium hydroxide (4 mL) that results in dark yellow colour. Phenol was detected by using Ferric chloride. Keller-Kiliani test was used for the detection of glycosides. Formation of froth upon vigorous shaking of diluted sample shows the presence of saponins. For tannins detection, Lead acetate test was performed which gave cream yellow colour. For the detection of terpenes, Copper acetate and Salkowai's test were used.

ACETIC ACID INDUCED WRITHING

The procedure was similar as described by Brandão et al. (2013) and Khan and Gilani (2010). The acetic acid was used to induce pain in mice by i.p. injection (0.7% v/v acetic acid 0.1 mL/kg), 30 min after the administration of plant extract (10-100 mg/kg) and standard drug (diclofenac sodium). All mice were individually placed in transparent cage. After 5 min, the number of writhes (abdominal stretching and extension of at least one hind limb) was counted for 20 min and compared with control group (normal saline 10 mL/kg, i.p.).

HOT PLATE TEST

After administration of test (Ce.Cr 10, 30 and 100 mg/kg) and standard drug (Tramadol 30 mg/kg) the mice were individually positioned on hot surface kept at 55°C and the latency to pain reaction (jumping or licking of paw) was observed during contact with hot surface. The latency period in seconds was noted before and after the drug administration after every 30 min for 2 h. The cut-off time was 20 s to prevent paw injury (Bukhari 2013; Raquibul Hasan et al. 2009). The increase in the latency period in seconds of test drug treated animals compared with the values of the control animals received normal saline (10 mL/kg, i.p.).

TAIL IMMERSION TEST

After dividing in five different groups the tail of individual mouse was marked (5 cm). The normal saline (10 mg/kg), test (10, 30, 100 mg/kg of plant extract) and standard drug (tramadol 30 mg/kg) was given an i.p. injection. After 30 min of injection, the marked area (5cm) of tail was dipped in water maintained at 55°C. The tail withdrawal time in seconds at 0, 30, 60, 90 and 120 min was taken as the response time, with a cut-off time of 15 s to prevent any injury (Janssen et al. 1963; Toma et al. 2003).

STATISTICAL ANALYSIS

All experimental results were expressed as mean \pm standard error of mean (SEM) with five animals in each group. Data were analyzed via one-way ANOVA between different test groups followed by Tukey post-hoc test. The results were considered statistically significant at $p < 0.05$. All graphs were generated by Graphpad Prism Software (GraphPAD, San Diego, CA, USA).

RESULTS

PHYTOCHEMICAL ANALYSIS

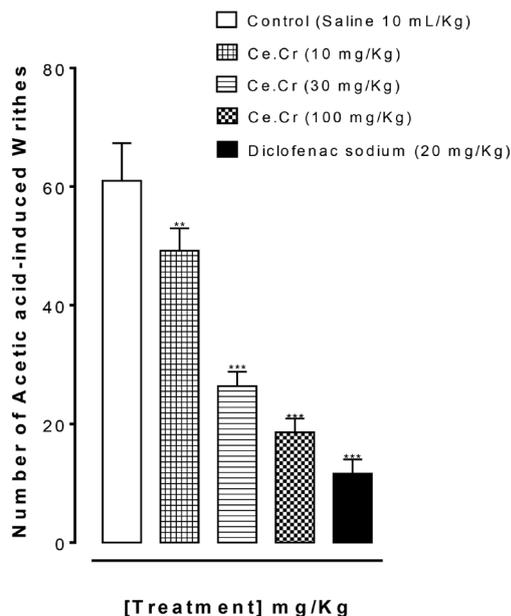
The phytochemical analysis showed positive results for the presence of alkaloids, flavonoids, glycosides, phenol, saponins, tannins and terpenoids.

EFFECT ON ACETIC ACID MEDIATED WRITHINGS

Ce.Cr dose-dependently (10, 30 and 100 mg/kg) decreased number of writhes evoked by acetic acid (Figure 1). The number of writhes in control saline group was 61 ± 2.82 . Ce.Cr at the doses of 10, 30 and 100 mg/kg reduced number of writhes to 49 ± 1.68 ($p < 0.01$ vs. saline group), 26 ± 1.07 and 18 ± 1.03 ($p < 0.001$ vs. saline group), respectively. Diclofenac sodium (20 mg/kg) decreased number of writhes to 11 ± 1.07 ($p < 0.001$ vs. saline group).

EFFECT ON HOT PLATE TEST

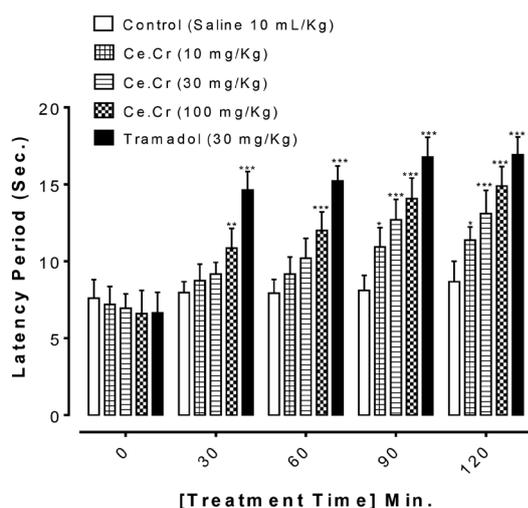
Ce.Cr at doses of 10, 30 and 100 mg/kg prolonged the latency time against thermal pain generation. The latency times of control saline group animals at 0, 30, 60, 90 and 120 min were 7.61 ± 0.54 , 7.95 ± 0.32 , 7.92 ± 0.40 , 8.11 ± 0.43 and 8.67 ± 0.59 , respectively. The latency time of Ce.Cr (10 mg/kg) treated group at 0, 30, 60, 90 and 120 min were 7.19 ± 0.52 , 8.75 ± 0.47 , 9.16 ± 0.49 , 10.92 ± 0.56 ($p < 0.05$ vs. saline group) and 11.37 ± 0.42 ($p < 0.05$ vs. saline group), respectively. The latency time of Ce.Cr (30 mg/kg) treated group at 0, 30, 60, 90 and 120 min were 6.95 ± 0.41 , 9.16 ± 0.34 , 10.20 ± 0.57 , 12.69 ± 0.59 ($p < 0.001$ vs. saline group) and 13.11 ± 0.67 ($p < 0.001$ vs. saline group), respectively. The latency time of Ce.Cr (100 mg/kg) treated group at 0, 30, 60, 90 and 120 min were 6.59 ± 0.67 , 10.85 ± 0.57 ($p < 0.01$ vs. saline group), 12 ± 0.90 ($p < 0.001$ vs. saline group), 14.06 ± 0.60 ($p < 0.001$ vs. saline group) and 14.89 ± 0.56 ($p < 0.001$ vs. saline group).



Values shown are mean \pm SEM, $n=5$. ** $p<0.01$, *** $p<0.001$ vs. saline group, one-way analysis of variance with post-hoc Tukey test

FIGURE 1. Bar chart showing inhibitory effect of the *Caralluma edulis* crude extract (Ce.Cr) and diclofenac sodium on the acetic acid-induced writhes in mice

group), respectively. The latency time of tramadol (30 mg/kg) treated group at 0, 30, 60, 90 and 120 min were 6.64 ± 0.59 , 14.62 ± 0.53 ($p<0.001$ vs. saline group), 15.22 ± 0.43 ($p<0.001$ vs. saline group), 16.77 ± 0.57 ($p<0.001$ vs. saline group) and 16.92 ± 0.51 ($p<0.001$ vs. saline group), respectively, as shown in Figure 2.

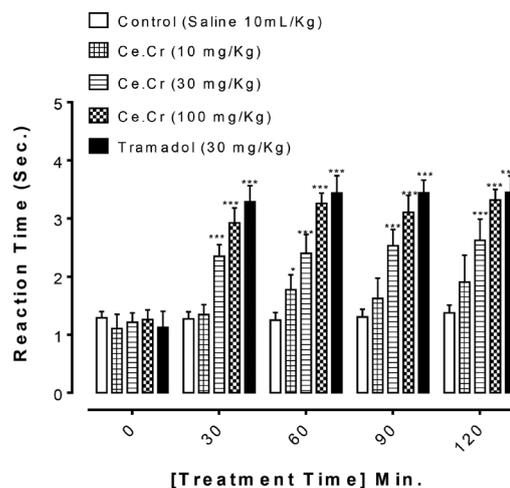


Values shown are mean \pm SEM, $n=5$. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. saline group, one-way analysis of variance with post-hoc Tukey test

FIGURE 2. Bar chart showing effect of the *Caralluma edulis* crude extracts (Ce.Cr) and tramadol on the latency time of mice in hot plate test model

TAIL IMMERSION

Ce.Cr at the doses of 10, 30 and 100 mg/kg prolonged the reaction time against thermal stimuli. The withdrawal time of control saline group animals at 0, 30, 60, 90 and 120 min. were 1.29 ± 0.10 , 1.27 ± 0.05 , 1.25 ± 0.05 , 1.30 ± 0.06 and 1.38 ± 0.05 , respectively. The withdrawal time of Ce.Cr (10 mg/kg) treated group at 0, 30, 60, 90 and 120 min were 1.11 ± 0.10 , 1.35 ± 0.07 , 1.77 ± 0.11 ($p<0.05$ vs. saline group), 1.62 ± 0.15 and 1.91 ± 0.20 ($p<0.001$ vs. saline group), respectively. The withdrawal time of Ce.Cr (30 mg/kg) treated group at 0, 30, 60, 90 and 120 min were 1.21 ± 0.07 , 2.35 ± 0.08 ($p<0.001$ vs. saline group), 2.40 ± 0.14 ($p<0.001$ vs. saline group), 2.53 ± 0.12 ($p<0.001$ vs. saline group) and 2.62 ± 0.16 ($p<0.001$ vs. saline group), respectively. The withdrawal time of Ce.Cr (100 mg/kg) treated group at 0, 30, 60, 90 and 120 min were 1.26 ± 0.07 , 2.92 ± 0.11 ($p<0.001$ vs. saline group), 3.26 ± 0.07 ($p<0.001$ vs. saline group), 3.11 ± 0.13 ($p<0.001$) and 3.32 ± 0.08 ($p<0.001$ vs. saline group), respectively. The withdrawal time of tramadol (30 mg/kg) treated group at 0, 30, 60, 90 and 120 min were 1.13 ± 0.12 , 3.28 ± 0.12 ($p<0.001$ vs. saline group), 3.44 ± 0.13 ($p<0.001$ vs. saline group), 3.44 ± 0.09 ($p<0.001$ vs. saline group) and 3.45 ± 0.12 ($p<0.001$ vs. saline group), respectively, as shown in Figure 3.



Values shown are mean \pm SEM, $n=5$. * $p<0.05$, *** $p<0.001$ vs. saline group, one-way analysis of variance with post-hoc Tukey test

FIGURE 3. Bar chart showing effect of the *Caralluma edulis* crude extracts (Ce.Cr) and tramadol on the reaction time of mice in Tail immersion model

DISCUSSION

In this investigation, analgesic activity was evaluated through acetic acid-induced writhing, tail immersion and hot plate methods in mice. The intraperitoneal injection of acetic acid releases the endogenous mediators such as bradykinin, prostaglandins (PGE_2 , $PGE_2\alpha$) and cytokines ($IL-1\beta$, $IL-8$ and $TNF-\alpha$). These products increase in peritoneal fluid and cause swelling and pain that stimulate

the nociceptive neurons. Non-steroidal anti-inflammatory drugs (NSAIDs) cause inhibition of these chemical mediators and reduce pain sensations (Afsar et al. 2015; Ferreira et al. 2013; Santa-Cecília et al. 2011). The plant extract was prominently reduced the acetic acid-induced writhing in a dose dependant manner (10, 30 and 100 mg/kg) and comparable with standard drug diclofenac sodium. These finding recommend that the Ce.Cr extract have peripheral analgesic activity and their mechanism of action might be due to inhibition of prostaglandins synthesis.

Thermal nociception models (hot plate and tail immersion) were used to assess the central activity of plant extract (Vilela et al. 2009). Ce.Cr in dose-dependent manner (10, 30 and 100 mg/kg) prolonged the latency period in hot plate provoked by heat stimuli, that showed significant analgesic effect. In tail immersion method the latency for the tail withdrawal response was also increased, similarly as caused by standard analgesic drug tramadol, indicating the central analgesic effect of *Caralluma edulis*. The result of plant extract was found that the Ce.Cr acts both peripherally and centrally.

The anti-nociceptive effect of plant may be due to the presence of flavonoids and tannins, as these phytochemicals are known for their analgesic effect (Zulfiker et al. 2010).

CONCLUSION

Caralluma edulis showed a significant analgesic activity against chemical and thermal nociception models. These findings showed that plant extract possess both peripheral and central analgesic activity. Further evaluation shall be made on possible mechanism of action and also for identification, characterization of active compounds responsible for analgesic activity.

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