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Full Length Research Paper

Possible mode of action of *Cissus quadrangularis* in experimental induced nociception in mice

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The methanolic root extract of *Cissus quadrangularis* L. (CQ) belongs to the family Vitaceae and was evaluated for its antinociceptive activity by radiant heat and acetic acid induced pain models in mice, at four different dose levels of 10, 50, 100 and 200 mg/kg by intraperitoneal (i.p.) route. The results obtained in the writhing test showed that CQ at a dose levels of 50, 100, 200 mg/kg significantly inhibited the acetic acid-induced writhing in mice in a dose dependant manner decreasing the writhes by 84.23% at a dose of 200 mg/kg. In the tail-flick assay, the results obtained with the intraperitoneal administration of CQ at dose levels of 50, 100, 200 mg/kg i.p. demonstrated significant antinociceptive activity increasing the latency of tail flick in mice comparable to the control drug, pentazocine (10 mg/kg i.p.). The intraperitoneal administration of naloxone along with CQ decreased the latency of tail flick in mice, which suggest that CQ contain antinociceptive substances which appear to be related to the activation of opioid receptors. The results showed that the methanolic extract of *C. quadrangularis* L. had excellent antinociceptive activity.

Key words: *Cissus quadrangularis*, antinociceptive, spinal algesia.

INTRODUCTION

Cissus quadrangularis L (CQ) (Family: Vitaceae) commonly called as Hajoda is one of the most widely used ingredients in alternative medicine (Ayurveda) for the treatment of piles, anorexia, indigestion, chronic ulcers, asthma, otorrhoea, wounds and in augmenting fracture healing process (Kirtikar and Basu, 1999; Agarwal, 1997; Rajpal, 2002). The intramuscular administration of the alcoholic extract of this plant has been reported to facilitate healing of fractured bones in albino rats (Udupa et al., 1965). *C. quadrangularis* has also been reported for sedative and anticonvulsant activity (Ngo Bum et al., 2008) and our previous study has shown that CQ possesses neuropharmacological effects (Vishwanatha et al., 2006). The phytochemical studies reveal the presence of known flavonoids such as quercetin and kaempferol along with resveratrol, piceatannol, pallidol, ascorbic acid, ketosteroid, carotene and Stilbene derivetives (Sen, 1966; Saburi et al., 1999 Adesanya et al., 1999). It is well known that flavonoids are some of the most widely spread phenolic compounds in the plant world and have a range of biological activities and pharmacological effects including a pronounced anti-inflammatory, analgesic and anticonvulsant effects (Shshidi et al., 1998; Alcaraz and Ferrandiz, 1987; Ngo Bum et al., 2008).

C. quadrangularis is one of them. Roots of medicinal plants are common ingredients of many folk and herbal medicines (Kweifio-Okai, 1991) and root extracts of a number of medicinal plants have been reported to possess pharmacological activity, mainly analgesic and anti-inflammatory activity (Sen et al., 1993; Sen and Nag Chaudhuri, 1991, Panthong et al., 2007). Hence, in the present study an attempt has been made to carry out a preliminary investigation of the antinociceptive activity and possible mechanism of action of roots of CQ in mice.

MATERIALS AND METHODS

Plant material

Fresh roots of CQ were collected in the month of September from botanical garden of KLES's College of Pharmacy, Hubli. Diclofenac

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sodium and pentazocine were purchased from the local market. The roots were authenticated by Dr Ganesh R. Hegade, Head, Department of Botany, Karnataka University, Dharwad, Karnataka, India.

Preparation of extract

The roots were shade-dried, until free from moisture. Then, they were subjected to size reduction to get coarse powder of desired particle size. 1 kg of the powdered material was soaked in methanol overnight at room temperature (20 to 30℃) with occa sional shaking.

The powdered material was subjected to successive extraction in a soxhlet apparatus using methanol. The resultant extract was stored in a desiccator prior to use.

Animals

Swiss albino mice (20 to 25 g) of either sex were used in the present investigation. They were given the standard laboratory diet; the animals were housed in an air-conditioned room (22 ± 1℃, relative humidity 50 ± 10%). A 12:12 light: dark cycle was followed and they were given free access to standard rat feed and water *ad libitum.* Guidelines for the care and use of the laboratory animals (National Institute of Health, USA) were followed during the experiment. The animal study was approved by institutional animal ethical committee.

Acute toxicity studies

Mice were divided into four groups of three animals each and CQ was injected intraperitoneally in doses of 5, 50, 300 and 2000 mg/kg. The LD_{50} (24 h) was calculated according to CPCSEA guidelines (up and down Method, OECD guide line No.425).

Acetic acid induced writhing

The mice were divided into six groups of six each. The first group served as control and received normal saline (0.1 ml/10 g i.p). The second group was administered diclofenac sodium (10 mg/kg p.o) as the standard drug and remaining groups received methanolic extract of CQ 10, 50, 100 and 200 mg/kg, i.p. resepectively. 30 min later, each mouse was given 0.1 ml /10 g i.p of 1% acetic acid ((Witkin et al.,1961). 5 min after acetic acid induction, the number of writhes like abdominal muscle contraction, stretching of the hind limbs and trunk twisting were counted for 15 min. The results were expressed in percentage of inhibition (Collierh et al., 1968).

Tail flick method

The tail flick test was performed as described by D'Armour and Smith (1941) using analgesiometer. The animals were exposed to noxious stimulus (that is, radiant heat) and tail flick latencies (the time required for the flicking of tail, that is, the reaction time) and a mean of two pre–drug recordings were taken as basal value (0 min), in order to prevent tissue injury a cut-off time of 10 s (based on the reaction time, which generally varied between 9 and 10 s) were maintained. The animals not responding up to 10 s, the cut-off time was considered as the latency (Rambadran and Bansinath, 1986). Analgesic activity was expressed as the increase in response time with respect to the corresponding pre-treatment control. The mice were divided into seven groups of six each. The first group served as control and received normal saline (0.1 ml/10 g i.p). The second group was administered pentazocine (10 mg/kg, i.p.) as the standard drug and 3rd to 6th groups received methanolic extract of CQ (10, 50, 100 and 200 mg/kg, i.p.) resepectively. The 7th group received naloxone and 100 mg/kg of CQ. Naloxone was applied 15 min prior to administration of drugs and test substances (1 mg/kg, i.p.).

Statistical analysis

The results were expressed as the mean ± S.E.M. The results obtained from the present study were analyzed using one way ANOVA followed by Dunnett's multiple comparison tests. Data was computed for statistical analysis by using Graph pad prism software. *P* values less than 0.05 were considered to be significant.

RESULTS AND DISCUSSION

From the acute toxicity studies the LD_{50} of CQ was found to be 2000 mg/kg. In acetic induced writhing model, CQ showed significant analgesic activity. The methanolic extract of CQ at dose of 10 mg/kg did not show significant activity but CQ at dose levels of 50, 100, 200 mg/kg showed significant analgesic activity with a dose-dependent reduction in number of writhing with 44.50% ($p < 0.01$), 61.01% ($p < 0.01$) and 84.23% ($p < 0.01$) of inhibition respectively as compared to normal (Table 1). Maximum inhibition was observed at the dose of 200 mg/kg. Diclofenac (10 mg/kg) showed the most significant inhibition (peripheral action). The acetic acid induced writhing is a visceral pain model and has been associated with release of arachidonic acid via cyclooxygenase, bradykinins and substance P. Prostaglandins (PGE_2 and $PGF_{2\alpha}$) biosynthesis plays an important role in nociceptive mechanism (Baird-Lambert and Jamieson, 2007). Several non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac block the synthesis of prostaglandins by inhibiting cyclooxygenase catalyzed peroxidation of arachidonic acid. Previous report suggests that many plant extracts possess both analgesic and anti-inflammatory properties which act by inhibition of cyclooxygenase (Morteza-Semnani et al., 2002). It is also reported that certain molecules which have flavonoidic, alkaloidic or tannic structure had an anti inflammatory activity (Mavar-Manga et al., 2004, Hoult et al., 1994). Analgesic effects of methanolic extract of CQ may involve cyclooxygenase inhibition by flavonoids.

In this study, intraperitoneal administration of CQ extract resulted in significant prolongation of the response latency in the tail flick method. CQ at a dose of 50, 100, 200 mg/kg increased the tail flick latency significantly in a dose-dependent manner. The effects reach a peak at approximately 60 min after administration and then gradually decreased. CQ at a dose of 100 and 200 mg/kg increased the tail flick latency to 3.37 ± 0.21 ($p < 0.05$) and 6.62 ± 0.26 ($p < 0.05$) s at 30 min respectively as compared to control 2.33 ± 0.14 s. At 60 min, response latency was found to be 4.42 ± 0.38 ($p <$

Table 1. Analgesic effect of CQ on acetic acid-induced nociception in mice ("writhing test").

Treatment	No. of writhes in 15 min	Percentage inhibition (%)
Normal saline (Control)	34.83 ± 0.60	-
CQ (10 mg/kg)	33.75 ± 0.46^{ns}	3.10
CQ (50 mg/kg)	19.33 ± 1.44**	44.50
CQ (100 mg/kg)	13.58 ± 1.83**	61.01
CQ (200 mg/kg)	5.49 ± 0.63**	84.23
Diclofenac (10 mg/kg)	2.16 ± 1.00**	93.79

Values are mean ± S.E.M; n = 6 in each group. **, $p < 0.01$, ^{ns}, non-significant when compared to control.

Table 2. Analgesic effect of CQ on radiant heat induced nociception in mice ("tail flick test").

Treatment	Tail flick latency in seconds			
	0 min	30 min	60 min	120 min
Normal saline (Control)	2.31 ± 0.16	2.33 ± 0.14	2.54 ± 0.16	2.53 ± 0.13
Pentazocine (10 mg/kg)	2.34 ± 0.26^{ns}	7.04 ± 0.22**	9.58 ± 0.26**	7.28 ± 0.23**
CQ (10 mg/kg)	2.16 ± 0.16^{ns}	2.36 ± 0.14 ^{ns}	2.61 ± 0.18 ^{ns}	2.71 ± 0.21 ^{ns}
CQ (50 mg/kg)	2.33 ± 0.21^{ns}	2.89 ± 0.23 ^{ns}	4.42 ± 0.38**	3.29 ± 0.26 ^{ns}
CQ (100 mg/kg)	2.38 ± 0.18^{ns}	3.37 ± 0.21*	5.89 ± 0.42**	3.95 ± 0.24**
CQ (200 mg/kg)	2.41 ± 0.14^{ns}	6.62 ± 0.26**	7.21 ± 0.36**	5.10 ± 0.29**
CQ (100 mg/kg) + Naloxone (1 mg/kg)	2.32 ± 0.13^{ns}	2.86 ± 0.27 ^{ns}	3.86 ± 0.31*	2.56 ± 0.23 ^{ns}

Values are mean ± SEM; n = 6 in each group. ^{ns} non-significant, *$p < 0.05$, ** $p < 0.01$ compared to normal.

0.01), 5.89 ± 0.42 ($p < 0.01$), 7.21 ± 0.36 ($p < 0.01$) s for 50, 100, 200 mg/kg of CQ respectively as compared to control 2.54 ± 0.16 s. Pentazocine (centrally action) at a dose of 10 mg/kg produced most significant activity with tail flick latencies of 7.04 ± 0.22, 9.58 ± 0.26 and 7.28 ± 0.23 s at 30, 60 and 120 min respectively. The tail flick test is one of the widely used animal models to test the analgesic compounds acting centrally. In this model, thermal algesia is induced by radiant heat which involves spinal cord and higher centres. This model is considered selective for opioid-like analgesic compounds. CQ produced a significant analgesic activity in this model suggesting its central analgesic activity. These results were in agreement with our previous study where CQ produced analgesic activity in hot plate test showing its central analgesic activity (Vishwanatha Swamy et al., 2006).

Antinociceptive activity of CQ was partially antagonized by pretreatment with naloxone in tail flick test. Naloxone, an antagonist of membrane bound opioid receptors δ (OP1), κ (OP2) and μ (OP3), was also employed in an attempt to explore the possible mechanism involved in the antinociceptive effects of CQ. From Table 2, it can be observed that the antinociceptive effect induced by methanolic extract of CQ (100 mg/kg) was partially antagonized by pretreatment with naloxone. The tail flick latency of CQ 100 mg/kg alone was found to be 3.37 ± 0.21 and 5.89 ± 0.42 s at 30 and 60 min, respectively as compared to normal. But when CQ was given along with naloxone, analgesic effect was found to be decreased. Tail flick latency was found to be 2.86 ± 0.27 and 3.86 ± 0.31 s. These results suggest that CQ contains antinociceptive substances that appear to be related to activation of opioid receptors.

Antinociceptive effect observed in both these experiments with CQ extract indicates the involvements of both peripheral and central mechanisms. The analgesic effect is reduced partially after naloxone treatment; some of the other nonopioid mechanisms may also be involved. It may be possible that CQ may modulate some other neurotrasmitters/neuromodulaters involved in the regulation of pain sensitivity. Recently, Khanna et al. (1995) suggested the involvement of neurotransmission in antinociceptive effect of *Azadirachta indica* leaf extract. The presence of flavonoid, quercetin (Rajnarayana et al., 2001) and other unidentified flavonoids might account for the antinociceptive actions of CQ. In conclusion, the present study showed that the methanolic extract of CQ had antinociceptive activity in both the models of noxious stimuli induced by radiant

heat (tail flick) and acetic acid induced writhing models.

Further biological and phytochemical studies are suggested to determine the active constituents responsible for analgesic activity.

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