RESEARCH ARTICLE

Evaluation of anti-nociceptive activity of *Cissus quadrangularis* on albino mice

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Throughout the history, man has used several forms of therapy for relief of pain; among them, medicinal herbs have gained popularity because of its wide use and less side effects. *Cissus quadrangularis*, for example, is a commonly used folklore medicine in India to hasten the fracture healing process; in Cameroon, the whole plant is used for the treatment of oral dehydration, while in Africa and Asia, the leaves, stems and roots are utilized for the treatment of various ailments. The plant extract were prepared by using chloroform and distilled water in proportion (20:80), macerated for 72 h with occasional stirring and concentrated under dry vacuum. The extract of *Cissus quadrangularis* was examined for centrally acting analgesics by using the hot plate method, formalin test and acetic acid-induced writhing method for peripherally acting analgesics. The doses administered were 250 mg/kg and 350 mg/kg. The animal that administered both the doses showed significant analgesic activity. The animal that administered a dose of 350 mg/kg has shown the maximum analgesic activity which is comparable to the standard.

Key words: Analgesic, *Cissus quadrangularis*, formalin, writhing

INTRODUCTION

In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 5 year period. Pain is sensiorial modality, which in many cases represents the only symptom for diagnosis of several diseases. It often has a protective function throughout out history and man has used several therapies for the management of pain.[1] Medicinal herbs are highly highlighted due to their wide use and less side effects. An example is *Papaver somniferum*, from which morphine was isolated. It is regarded as a prototype of opiate analgesic drugs. For the relief of pain, opiates generally acts on the central nervous system, exercising their effects through three receptors (μ, κ and δ); such drugs are specially important for the treatment of chronic pain. Although morphine has reigned for centuries as the king of pain killers, its rule cannot be considered as totally benign. There are concerns regarding the side effects and addictive properties, which include respiratory depression, drowsiness, decreased gastrointestinal motility, nausea and several alterations of endocrine and autonomic nervous system.[2] Therefore, the currently used analgesics such as opiates and non-steroidal anti-inflammatory drugs are not useful in all cases; therefore, there arises the requirement for a medicinally active plant. The plant *Vitis* or *Cissus quadrangularis* (Sanskrit - Asthishrinkhala, Vajravalli; Hindi - Harjor) belongs to the family Vitaceae and has been used as antihelminthic, dyspeptic, digestive tonic, analgesic in eye and ear diseases, scurvy, irregular menstruation, asthma,[3,4] fractures of bones and for complains of the back and spine.[5,6] The intramuscular administration of the alcoholic extract of this plant has been reported to facilitate the healing of fractured bones in albino rats.[7,8] The methanolic extract of *Cissus quadrangularis* promoted the healing process of experimentally fractured radius-ulna of dogs, as evidenced by radiological and histopathological examinations.[9]

The objective of the present work is to evaluate the centrally as well as peripherally acting analgesic property of water: chloroform (80:20) extract of *Cissus quadrangularis* on albino mice which may lead to the preparation of morphine and NSAIDS (non-steroidal anti-inflammatory drugs)-like substances that are devoid of side effects.

MATERIALS AND METHODS

Fresh plant of *Cissus quadrangularis* were collected from western Maharashtra in September 2006 and authenticated. The taxonomic identification of plant was performed by Dr. AK Magdum, H.O.D of Botany, Willingdon College, Sangli. The leaves were sun-dried and grinded and macerated in a mixture of chloroform and water (20:80) for 72 h with occasional stirring. The mixture was filtered and

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the filtrate was dried.

Acute Toxicity Study

Acute toxicity studies were carried out using acute toxic class method as per OECD guideline 425.[10] Acute toxicity for various plant extracts was measured by using groups of three Swiss albino mice by administering a dose 3000, 2000 and 1000 mg/kg in 12% Tween 80 orally and intraperitoneally; however, the control group received only 12% Tween 80. The groups were observed for mortality and behavioural changes during 48 h. The LD_{50} of *Cissus quadrangularis* in mice by oral and intraperitoneal routes was found to be 4 and 3.5 g/kg, respectively.[11]

Animals

Adult Swiss albino mice (both sex) weighing 25-30 g are used in these studies. The animals were maintained at 28 ± 2°C at a relative humidity of 50-55% and at a 12 h light and dark cycle. Animals were housed in groups of five per cage and had free access to food and water. All the animals were acclimatized to laboratory conditions prior to experimentation. All protocols for animal experiment have been approved by institutional animal ethical committee.

Phytochemical Screening

The extract were also screened with the help of chemical test like Lieberman Buchard, ferric chloride, copo of magnesium and Vanillinsulphuric acid tests for the presence of sterols, phenolic compounds, flavonoids and saponins, respectively.[12,13]

Analgesic Activity

Hot plate method

The method was originally developed by Woolfe and MacDonald. The paws of mice and rats are very sensitive to temperature at 55 ± 0.5°C, which are not damaging to the skin. The response is in the form of jumping, withdrawal of the paws or the licking of the paws.[14] The animals were placed on Eddy's hot plate maintained at a temperature of 55 ± 0.5°C. A cut-off period of 15 s was observed to avoid damage to the paw. Reaction time and the type of response were noted using a stopwatch. Control mice were treated with a vehicle (12% Tween 80, 1 ml/100 gm of body weight). Pentazocine was used as positive control (10 mg/kg) which was administered i.p. The extracts of *Cissus quadrangularis* (250 and 350 mg/kg) were intraperitoneally administered in the test group. Each group contained five animals.

Formalin Test

The negative groups were treated as previously indicated. While positive control received 10 mg/kg of pentazocine orally, the two other groups orally received the following doses: 250 and 350 mg/kg. Half an hour after the treatment, to negative and positive received 0.05ml of 10% formalin into the right hind paw. In the test group, formalin is administered after 1 h. The duration of paw licking, which is an index of nociception, was recorded in two different time series: the first 5 min (neurogenic pain) and the duration between the fifteenth and the thirteenth minute (inflammatory pain) after formalin administration.[15,16]

Writhing Test

Animals were divided in into four groups containing five animals in each group. Group 1 served as a control and treated with (12% Tween 80, 1 ml/100 gm of body weight). The second and third groups received the extract of *Cissus quadrangularis* with doses of 250 and 350 mg/kg. The fourth group served as a positive control and it received standard indomethacin with a dose of 10 mg/kg. One hour after administration, each animal intraperitoneally received 1% acetic acid with a volume of 1 ml/100 g body weight. After the administration of acetic acid injection, the number of stretching or writhing responses per animal was recorded during the subsequent 10 min.[17]

Statistical Analysis

The statistical analysis of all the results was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. All the results obtained in the study were compared with the vehicle control group. P values < 0.05 were considered to be statistically significant.

RESULTS

Acute Toxicity

The results showed no clinical signs and mortality of the animal; therefore, an LD50 > 3000 mg/kg body weight may be assumed.

Phytochemical Analysis

The phytochemical analyses of *Cissus quadrangularis* reveal a high content of ascorbic acid, carotene, phytosterol substances and calcium; moreover, there have also been reports of the presence of sitosterol, amyrin and amyrone.[15]

Analgesic Activity

Hot plate method

The extract of *Cissus quadrangularis* when intraperitoneally administered with a dose of 350 and 250 mg/kg in mice has shown significant analgesic activity in hot plate method as supported by increase in latency time. The increase in latency time is dose dependant.

Both the doses of the extract have shown significant analgesic activity; however, it is the maximum for the dose of 350 mg/kg and this is comparable with standard.

Table 1: Effect of Cissus quadrangularis extract administered intraperitoneally on the latency of mice exposed to hot plate

Groups	Dose mg/kg	Mean latency before and after drug administration(s) ± S.E.M.				Percentage inhibition		
		0 min	30 min	60 min	90 min	30 min	60 min	90 min
Control.	Vehicle	3.8 ± 0.37	4.2 ± 0.3	4.2 ± 0.5	3.8 ± 0.48	-	-	-
Pentazocine	10	4 ± 0.31	10 ± 0.7**	13.8 ± 0.3**	14.2 ± 0.37**	60.00	71.01	71.83
C. quadrangularis extract.	350	4.2 ± 0.7	9 ± 0.31**	12.5 ± 0.5**	13.2 ± 0.37**	53.33	66.4	68.18
C. quadrangularis extract	250	4.4 ± 0.4	7.4 ± 0.5**	10.2 ± 0.56**	12.2 ± 0.44**	40.54	56.86	63.93

n = 5 the values are expressed in Mean ± SEM; ** = $P < 0.05$ when compared with control group

Table 2: Anti-nociceptive effect of *Cissus quadrangularis* extract on formalin test in mice

Groups	Dose	First phase(0-5 min)		Second phase (15-30 min)	
		Mean licking time. SEM	Percentage inhibition	Mean licking time SEM	Percentage inhibition
Control	Vehicle	60 ± 0.89	0.00	25.6 ± 1.9	0.00
Pentazocine	10	14 ± 0.3**	76.66	3.8 ± 0.37**	85.15
C. quadrangularis extract	350	17.2 ± 0.82**	71.33	4.2 ± 0.58**	83.59
C. quadrangularis extract	250	25.4 ± 1.4**	57.66	4.8 ± 0.58**	81.25

n = 5 the values are expressed in Mean ± SEM; ** = $P < 0.05$ when compared with control group

The increase in the latency time and % pain inhibition is summarized in Table 1.

Formalin Test

The extract of *Cissus quadrangularis* significantly reduced the licking time for both the doses. The extract inhibited the first phase (neurogenic pain) and as well as the second phase (inflammatory pain). The data of formalin test and Percentage protection is shown in Table 2

Writhing Test

The oral administration of *Cissus quadrangularis* extract significantly inhibited the writhing reaction induced by acetic acid. Both the doses of the extract inhibit acetic acid-induced writhing; however, the dose of 350 mg/kg significantly reduced the writhing reaction. The data of writhing test is presented in Table 3.

Table 3: Anti-nociceptive effect of oral administration of *Cissus quadrangularis* extract on pain induced by intraperitoneal injection of acetic acid in mice

Groups	Dose (mg/kg)	Number of contractions	Percentage inhibition
Control	Vehicle	33 ± 0.54**	0.00
C. quadrangularis extract	350	13 ± 0.4**	60.60
C. quadrangularis extract	250	17.2 ± 0.66**	47.87
Indomethacin	10	9 ± 0.7**	72.72

n = 5 the values are expressed in Mean ± SEM; ** = $P < 0.05$ when compared with control group

DISCUSSION

Pain is a subjective experience, which is difficult to define exactly even though we all experience it. Pain is distinguished as two types, peripheral or neurogenic pain may involve the following pathological states: peripheral nociceptive afferent neurons which are activated by noxious stimuli and central mechanism which is activated by afferent inputs pain sensation.[18] The hot plate method is considered to be selective for screening of the compound acting through the opoid receptor; the extract of *Cissus quadrangularis* increased the mean basal latency which shows that extract act through centrally acting analgesics. The intraperitoneal injection of acetic acid produces pain through the activation of chemosensitive nocireceptor or irritation of the visceral surface, thereby leading to the liberation of bradykinins, histamine, prostaglandins and serotonins. Thus, the extract has inhibited the pain induced by acetic acid which indicates that plants act through both mechanisms, i.e. central as well as peripheral analgesics.[16,19]

Formalin produces pain through two phases: neurogenic pain releasing substance P and inflammatory pain with the release of serotonins, histamine bradykinins and prostaglandins.

Narcotic analgesic inhibits both the types of pain, while NSAIDS such as paracetamol aspirin inhibit only the peripheral pain.[20,21] The extract of *Cissus quadrangularis* has inhibited both the phases of pain induced by formalin which suggests that the extract may act as a narcotic analgesic. From the obtained pharmacological data, it is evident that the plant is having high potential of analgesic property. The analgesic activity is found to be dose dependant. The analgesic activity may be due to the presence of carotene, phytosterol substances, calcium, sitosterol, amyrin and amyrone.

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