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Analgesic , anti-inflammatory and antipyretic activity of *Cissus quadrangularis*

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Abstract

This study was intended to evaluate the analgesic anti-inflammatory and antipyretic activity of ethanolic extract of *Cissus quadrangularis* in experimental standard modals i.e. albino rats following oral administration. The results showed that the ethanolic extract significantly reduce the edema induced by carrageenan within 1 to 5 hrs. post dosing at all the dose levels used. On the analgesic property acetic acid induce writhing was significantly reduce in the formalin test, the extract also significantly decreases the painful stimulus in both phases of test which confirms central and peripheral effects of the drugs. Its effects on antipyretic activity were also appreciable it significantly reduces fever at higher doses within 2 hrs. on yeast induce hyperthermia in rats

Keywords: *Analgesic, Anti-inflammatory, Antipyretic, Cissus quadrangularis, albino rats*

Introduction

Cissus quadrangularis is the most common species, belonging to the family Vitaceae, commonly known as "Hadjod" in Hindi or bone setter due to its bone fracture healing property (Prasad et al.,1964). It is fleshly cactus like, jointed climber, distributed throughout hotter parts of India, also cultivated in garden. The total alcoholic extract of this plant, on parenteral administration, neutralizes the anti-anabolic effects of the cortisone in healing of fractures. The extract of the plant exhibit cardiotonic and androgenic property. Alcoholic extract of the stem showed activity against Escherichia coli (George et al.,1947; Oliver 1983) The leaf extract showed antifungal activity (Misra et al., 1949). The plant contains various secondary metabolites as previously reported (Sen 1996, 1964; Adesanya et al.,1999). Since medicinal plants are also use to treat burns, swelling and malaria, one can expect that they might possess analgesic anti-inflammatory and antipyretic activities as well. The search of these agents are largely concentrated on lower plants, fungi, bacteria (Fabry et al 1998). Less research focuses on higher plants although plants compounds such as berberine, emetine, sanguinarine having specialized uses have been isolated (Van Wyk et al 1977)

The paper reports on preliminary screening for the analgesic anti-inflammatory and antipyretic activities of *Cissus quadrangularis.*

Material and method

Plant Material

The plant material (*Cissus quadrangularis*) which is used for study was collected in august 2007 and was identified by the taxonomist of depts., Department of Botany, University of Rajasthan Jaipur. The voucher number was RUBL 20253. The material was dried under shade and powdered with the

mechanical grinder and stored in airtight container. The dried powdered material was extracted with 80% ethanol in a soxhlet apparatus (yield- 8.34%). Photochemical screening of the extracts revealed the presence of flavonoids, coumarins, steroids etc.

Animals

Inbred albino rats weighing 180-200 gm were used for the present experiment. The animals were maintained under standard husbandry conditions in the animal house of depts. Of zoology, centre of advance studies, university of Rajasthan Jaipur at temperature 25± 2˚C in the natural light and dark cycle and had free access to standard rat pallet diet (Ahsirwad food industries, Chandigarh, India) and water *ad libitum*

ANALGESIC STUDY

Analgesic effects was evaluated using three different models: the writhing test, tail flick test and formalin test

WRITHING TEST

Male Swiss rats (180-200 g) were used according to the method described previously by Koster et al. (1959). The total number of writhings , following intraperitoneal (i.p.) administration of 0.6% acetic acid , was recorded for 20 min. starting 10 min. after injection. The animals were pretreated with hydroalcoholic extracts (HAEs) from *C. quadrangularis* 50,100, 150 mg/kg b.wt respectively.

TAIL-FICK TEST

The basal reaction time of each mouse was determined using tail-withdrawal response when one-third of the tail was immersed in water bath at 51˚C (Jansen et al., 1963). The cutoff time for immersion was 180 s. The reaction time was evaluated 30, 60, 90, 120 and 240 min after oral administration of extracts, distilled water or acetylsalicylic acid.

FORMALIN TEST

The method used in our study was similar to that described previously (Shibata et al., 1989).Twenty microliter of 5% formalin was injected subcutaneously into the right hind paw of mice. The time (in seconds) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 5 min after formalin injection (early phase) and 20–30 min after formalin injection (late phase). Plants extracts (0.5 and 1.0 g/kg, i.p.) were administered 60 min before formalin injection. Indomethacin (10 mg/kg, i.p.) was administered 30 min before formalin injection. Control group received the same volume of saline by oral administration

Anti-inflammatory

Carrageenan induced hind paw edema in rats

Paw edema was produced in rats by carrageenan following the methods of Winter et al.
(1962) respectively. Male rats weighing 100–120 g were divided into groups of six animals.
A volume of 0.05 ml of 1% carrageenan in normal saline solution (NSS) in 0.2M carbonate buffer was
injected intradermally into the plantar side of the right hind paw of the rat. Test drugs and vehicle were given 1 h prior to carrageenan injection. Paw volumes were measured using a plethysmometer (model 7150, Ugo Basile,Italy) before as well as 1, 3 and 5 h after carrageenan, injection. Results obtained were compared with those obtained from their

Analgesic activity

Table 1: WRITHING TEST

Group	Treatment	n	Dose mg/kg	Route of adminis--tration	No. Of writhes	Inhibition Of Writhing Response (%)
I	Control	6	----	i.p.	48.06±4.04	-----
II	Aspirin	6	300	i.p.	10.05±2.12	79
III	*C.quadragularis*	6	50	i.p.	30.65±2.10	36
IV	*C.quadragularis*	6	100	i.p.	21.95±1.68	54
V	*C.quadragularis*	6	150	i.p.	9.68±1.84	79

Mean = S.E.M. of 6 animals.
** = P≤0.001= highly significant.
Group II ,III , IV, and V compared with Group I

Anti-inflammatory activity

Table2 Carrageenan –induced paw edema

Group	n	Dose(mg/kg)	Paw volume increase(ml)			Inhibition (%)		
			1hr	3hr	5hr	1hr	3hr	5hr
Control	6	-	0.36±0.07	0.69±0.05	0.82±0.03	-	-	-
Aspirin	6	300	0.10±0.02**	0.21±0.02**	0.27±0.03**	72	70	67
Cissus quadrangularis	6	50	0.22±0.04*	0.47±0.01*	0.51±0.02*	39	32	38
Cissus quadrangularis	6	100	0.18±0.03*	0.39±0.01*	0.42±0.04*	50	43	49
Cissus quadrangularis	6	150	0.07±0.01**	0.24±0.02**	0.35±0.02**	81	65	57

n = 6 animals in each group
* = p≤0.01 (significant)
** = p≤0.01(highly significant)

control groups, which received vehicle only

Antipyretic activity

Yeast induced hyperthermia in rats

In order to determine the antipyretic activity, rats treated with FCA (adjuvant-induced arthritis model) were employed (Yesilada and K¨upeli, 2002). The heat on the surface of left and right hind paws of each rat was measured every other day with a clinical contact digital thermometer (Prima long) and the difference between these two values was compared with that of control animals and results were evaluated statistically.

Result

Analgesic activity of Cissus quadrangularis

Administration of *Cissus quadrangularis* extract at the dose level of 50, 100 and

Antipyretic activity

Table3: Brewer's yeast induced pyrexia in rats

Group	n	Dose(mg/kg)	Rectal temperature in˚C at time (hr.)					
			-18[a] hr	0[b] hr	1 hr	3 hr	5 hr	6 hr
Control	6	-	38.65±0.27	39.20±0.04	39.91±0.11	39.76±0.14	39.64±0.07	39.83±0.06
Aspirin	6	300	38.47±0.25	39.32±0.13**	37.28±0.13**	36.11±0.08**	35.03±0.11**	36.03±0.21**
Cissus quadrangularis	6	50	38.13±0.12	39.41±0.12[ns]	39.31±0.12*	39.30±0.26[ns]	38.65±0.20[ns]	38.40±0.1[ns] 8
Cissus quadrangularis	6	100	38.25±0.14	39.63±0.10[ns]	38.08±0.15**	38.54±0.21[ns]	38.62±0.11[ns]	38.22±0.11[ns]
Cissus quadrangularis	6	150	38.68±0.09	39.48±0.14[ns]	38.24±0.09**	38.03±0.02**	38.03±0.08**	37.42±0.08**

n = 6 animals in each group
ns = non-significant
* = p≤0.01 (significant)
** = p≤0.01(highly significant)
a = temperature just before yeast injection
b = temperature just before drug administration

150 mg./kg b. wt. to the rats produced weak effect on the writhing induced by the injection of 0.6% acetic acid when compared with the aspirin (300mg/kg) by 79% while the treated group with *Cissus quadrangularis* inhibited the writhing by 36% ,54%, 79%

respectively(table 1). The ethanolic extract of *Cissus quadrangularis* (50-100 mg/kg) produced inhibition of formalin induce biphasic pain response (neurogenic and inflammatory pain) in

rats Fig 1. The analgesic effect of this fraction occurred predominately during the II phase; 100 mg dose level was more efficient in the late phase.

Anti-inflammatory activity of Cissus quadrangularis

The inhibitory activity on carrageenan induced rat hind paw edema, caused by the subplanatar administration of *Cissus quadrangularis*, at various assessment times after carrageenan injection are shown in table 2. aspirin, a cyclooxygenase inhibitor, at the dose of 300mg/kg body weight exhibited significant (p≤0.01) edema inhibition. *Cissus quadrangularis* at doses of 50,100,150 mg/kg boy weight also possessed significant (p≤0.001) inhibitory effect on carrageenan induced paw edema at all recorded times. This increase was observed at 1 hr. and was maximum at 5hr. after administration of carrageenan in the vehicle group.

Antipyretic activity of Cissus quadrangularis

The various serial extract of the *Cissus quadrangularis* produced a reduction ($p \leq 0.01$) in hyperpyrexia induced by dried yeast injection in rats, with activity being pronounced within 18 hrs. after administration of the extracts (table-3).

Discussion:

The inflammatory effect, analgesic, antipyretic properties of cissus quadrangularis ethanolic extracts were investigated in the present study The writhing test allows us to identify central and peripheral analgesic compound (Le Bars et al., 2001). The tail formalin test is recent algesiometric assay in which only behavior suggestive of pain is the licking of tail. The lack of 2 distinct phases after the administration of formalin in the tail may be due to a different pattern of the release of the chemical pain mediators at both the spinal and peripheral levels and this method mainly identifies peripheral analgesic (Koleniskov et al., 2004). The thermal model of the tail flick test is considered to be spinal reflex, but could also involved higher neural structures at this method identifies mainly central analgesic (Jensen and Yaksh 1986).

The extracts derived from *C.quadrangularis* exhibited analgesic activity in albino rats by inhibiting acetic acid induce writhing, which is a model of visceral pain (Vyklicky , 1979). Acetic acid induced writhing is a highly sensitive and useful test for analgesic drug development , but not a selective pain test as it gives false positives with sedatives , muscles relaxants and other pharmacological activities (Elisabetsky et al., 1995).

The analgesic property of *C.quadrangularis* can also probably be to the blockade of the effects or the synthesis and /or release of PGs and /or other endogenous substances that excite pain nerve endings (Panthong et al., 2007)

Although the writhing response test is very sensitive it has poor specificity as in analgesic screening test (Gupta and Verma 1991) therefore the formalin test and tail flick test were conducted to confirm and study the possible analgesic mechanism of the tested plants .The formalin test consist of 2 distinct phases that possibly reflecting different types of pain mechanisms. The I phase immediately after injection of formalin and last about 5 minutes this is due to result chemical, peripheral stimulation of nococeptors (Heapy et al., 1987). The II phase starts approximately 15-20 min after formalin injection and lasts for 20-40 min. the formalin test is sensitive to nonsteroidal anti-inflammatory drugs and other mild analgesics. In the study aspirin inhibited analgesic behavior during both the early and late phases.

The carrageenan test was selected because of its sentivity in detecting orally active inflammatory agents particularly in the acute phase of inflammation (Di Rosa et al., 1971; Di Rosa et al., 1972). The intraplatar injection the carrageenan in rats leads to paw edema. Its first phase that is 1 hour after infection results from the concomitant release of mediators, histamine, serotine and kinins on the vascular permeability. The IInd phase is correlated with elevated production of prostaglandins, oxygen derive free radicals & production of inducible cyclooxygenase (Panthong et al., 2004). Oral administration of the *Cissus quadrangularis* extracts suppressed that edematous response 1 hr after

carrageenan injection & this effect continues up to 5 hr It is well known that most of the anti-inflammatory analgesic drugs posses antipyretic activity *Cissus quadrangularis* revealed weak antipyretic effect at low dose i.e. 50mg/kg b.wt . but at higher dose i.e. 100 and 150mg/kg bwt. it produce marked antipyretic effect in brewel yeast induce fibril rats .In general , anti-inflammatory drugs produce their antipyretic action through inhibition of prostaglandin synthesis with in the hypothalamus (Clarke & Cumbi 1975; Zeil 1975). Although there is no direct evidence of *Cissus quadrangularis* to interfere with prostaglandin synthesis in hypothalamus but it can be supported by a related study in which *D. odorifera* extract was found to inhibit prostaglandin by synthesis (Goda et al., 1985) From these results is concluded that the extract from *Cissus quadrangularis* possess both peripheral and central analgesic activity along with marked antipyretic and anti-inflammatory activity in rats and also the present study provoke the traditional use of *Cissus quadrangularis* for the purpose of various ailments like analgesic, anti-inflammatory and antipyretic

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