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Research Article

POTENTIAL ANALGESIC EFFECT OF *CISSUS QUADRANGULARIS L.* AND *LEPEDIUM SATIVUM L.* ALONG WITH THEIR COMBINATION EXTRACTS

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ABSTRACT

The analgesic activity of methnolic root extract of *Cissus quadrangularis l.* (CQ) and seed extract of *Lepedium sativum l.* (LS) were studied in mice along with combination (CL) of CQ and LS at a ratio of 70:30. The analgesic effects were studied by using Tail flick and Hot plate method for tail and hand paw withdrawal time in mice. The extracts were analyzed at doses of 50,100 and 200 mg/kg i. p. The results suggest that the methnolic root extracts of CQ and LS contains some active principles, which possess analgesic activity. While the combination extract showed significantly potent tail flick withdrawal response in mice as compared to diclofenac sodium which was used as standard. Preliminary phytochemical evaluation was also carried out of these extracts. In phytochemical studies of methanol extract of CQ stem revealed the presence of triterpenes including α- amyrins, β- amyrins, β-sitosterol, ketosteroids, phenols, tannins, carotene and vitamin C in a considerable quantities.[1,2,8] While in methanol extract of seeds of LS phytochemical studies showed rich occurrence of primary metabolites such as, carbohydrate, protein, fatty acid and Vitamins: β-carotene, riboflavin, niacin and ascorbic acid, along with volatile oils, fixed oils, flavanoids, isothiocynate glycoside were detected when performed the phytochemical studies.[3,4] The analgesic activity of both the extracts and combination are found significant as compare to normal saline while the result of combination extract CL showed as potent as diclofenac sodium at dose of 50, 100 and 200 mg/kg.

Keywords: Cissus quadrangularis l (CQ); Lepedium sativum l (LS); Combination extract (CL).

INTRODUCTION

Cissus quadrangularis Linn (CQ) (Family-Vitaceae) is a medicinal and ornamental herb reputed to have great medicinal effects being use in traditional system of medicine. It is an edible plant which is distributed in Pakistan, India, Sri Lanka, Malaya, Java and West Africa[5].It is commonly known as "Hajdor" while in English it is called as "bone settler"; because of its ability to join bones. It has been reported that *Cissus quadrangularis l.* in fracture healing acts by stimulation of metabolism and increased uptake of the minerals, calcium, sulphur and strontium by the osteoblasts.[6,7]

It is one of the most widely used herbs in alternative medicine. It also involve as medicine for the treatment of piles, anorexia, indigestion, chronic ulcers, asthma, analgesic, wounds and in augmenting fracture healing process.[8,9,10] Paste of alcoholic extract of the plant was locally as well as intramuscularly facilitates rapid healing of fracture in albino rats.[11] Methanol extract possess analgesic, anti-inflammatory and venotonic effects associated with hemorrhoids. Its anti-inflammatory activity is due to flavonoids and sterols, luteolin and β-sitosterol respectively.[12]

Lepidium sativum l. (L.S)belongs to family Cruciferae (cabbage family) commonly known as "Common cress", "Garden cress" or "Halim". Plant is native to Pakistan and Morocco[6,7]. It is reported to exhibit antihypertensive,[13]

diuretic,[14] anti-inflammatory, analgesic, anticoagulant[15] antirheumatic[16] hypoglycemic[17] laxative, prokinetic,bantidiarrheal and antispasmodic properties[18]. It has also been shown to have anti-asthmatic and bronchodilatory potential in preliminary studies.[19, 20]

In traditional system of medicine and in folklore, these two plants have great potential as analgesic agent. More over modern therapy also utilizing their explored analgesic agent still awaiting for their extensive medicinal potential and their combined effects in various neurological disorders as phytoanalgesic agent. On this basis, we investigated the activity of the methnolic extract of the CQ and LS along with their combination CL for analgesic activity on mice which may be worthwhile to medical health.

MATERIALS AND METHODS

Collection and Identification

Fresh plant stems of *Cissus quadrangularis l.* were collected 3 kg from medicinal plants garden of Pharmaceutical Research Institute, Faculty of Pharmacy, University of Karachi, Karachi. While seeds of *Lepidium sativum l.* 2 kg was purchased by local herbal store, Karachi. The herbs are authenticated by Prof. Dr. Ghazala H. Rizwani samples were deposited in the herbal museum, Department of Pharmacognosy with LS and CQ specimen Nos. 100 and 101.

Preparation of plant extract

Fresh Plant CQ (3kg) washed and chopped into small pieces then percolated in methanol (Analytical Grade, Merck, Germany) for 15-30 days then filtrated through Whatman filter paper No.2. The filtrate was evaporated to dryness in getting extract on rotary vacuum evaporator (Iwaki, Tokyo) at controlled temperature and pressure and then followed same process twice. The same percolation procedure was followed for the seeds of LS (2Kg) in methanol. The combined methanolic extract (CL) was prepared by the addition of these two extract with ratio of 70:30 of CQ and LS respectively.

Phytochemical Screening

The Preliminary phytochemical studies were performed for qualitative evaluations of chemical compositions of these extracts. The extracts were used to determine the primary and secondary metabolites in them. [21, 22]

Animals

The pharmacological experiments were conducted using Swiss albino female mice weighing 20-25g. Animals were maintained under standard nutritional and environmental conditions with 12hr light/12hr dark cycle throughout the experiment. The animals were used after an acclimatization period of at least 5 days to the laboratory environment. The animals were deprived of food 24hr before experimentation. The animal ethical committee clearance was obtained from the institution for the present study.

Acute Toxicity Test

Mice were divided into 3 groups having ten animals in each group in which CQ, LS and CL were injected by i.p. respectively in doses from 50 to 2000 mg/kg. Deaths of animals were observed within 24 hr.

Analgesic Activity

Analgesic activity was performed by two ways; Tail Flick painful stimuli and Hot plate method. The mice were divided into five groups of six animals in each group. First group received normal saline (0.1 ml/10 g i. p.), Group second, third, and fourth received extract of CQ, LS and CL at doses of 50, 100 and 200 mg/kg, by i.p respectively. While group five received Diclofenic sodium (100 mg/kg i.p.) as standard drug.

Tail Flick Method

To evaluate the central analgesic effects of methanolic root CQ extract. Tail flick test was performed by time taken for mouse to withdraw the tail when immersed in hot water maintained at 55±0.5° C was measured.[23, 24]

Hot Plate Method

The analgesic activity of the extracts CQ, LS and CL were measured by hot-plate method[22]. The mice were placed on a hot plate maintained at 55±0.5°C. The reaction time was taken as the interval from the instant animal reached the hot plate until the moment animal licked its feet or jumped out. A cut off time of +10 s was followed to avoid any thermal injury to the paws. The reaction time was recorded before and after+30, +60,+90, and +120 min following administration of test or standard drug. [23, 24]

Statistical analysis

All the data obtained were expressed as Mean ± Standard Error Mean. Differences in means were estimated by means of ANOVA followed by Tukey HSD post hoc test. Results were considered significant at $P < 0.05$.

RESULTS

Acute Toxicity

In CQ and CL no acute toxicity and behavioral changes were observed while in acute toxicity and a general behavioral study of CL changes was found. The LD_{50} of the CL extract at dose of 1000 mg/kg by i.p. route in mice were observed continuously for any general behavioral changes and significant reduction of spontaneous locomotor motility, drowsiness and remarkably quiet were observed.

Phytochemical Screening

Phytochemical studies of CQ plant revealed that the presence of flavonols, quercetin and kaempferol along with resveratrol, piceatannol, pallidol, ascorbic acid, ketosteroid and carotene which are also reported by Saburi, 1999 and Sen, 1966. [25, 26]

The *Lepidium sativum* seeds contain volatile oil, active principle ami fatty oils, carbohydrate, proteins, along with different types of flavonoids, isothiocynates glycoside and Vitamins: β-carotene, riboflavin, niacin, and ascorbic acid.[21]

Analgesic Activity by Tail Flick Method

Analgesic activity at dose of 50mg/kg showed all extracts significant $p<0.05$ result, in respective to negative control i.e. normal saline. Analgesic activity at dose of 100mg/kg and 200mg/kg for all extracts were found significant $p<0.05$ analgesic activity, in respective to negative control i.e. normal saline. The combinations extract CL at all doses showed more potent result like standard Diclofenic sodium (100 mg/kg i.p.) as compared to C.Q and L.S.

Table 1: Analgesic Activity by Tail Flick Method (50 mg / kg)

Drug	Dose mg/kg	Time (Sec)				
		0	30	60	90	120
Control	0.3 ml	1.06±0.03	1.14±0.08	1.07±0.03	1.12±0.08	1.17±0.06
Standard	100	3.06±0.05	4.54±0.25	4.89±0.17	5.16±0.10	5.09±0.12
C.Q	50	2.63±0.23	3.32±0.24	3.6±0.16	3.88±0.06	3.91±0.17
L.S	50	2.44±0.13	2.88±0.05	3.51±0.20	3.83±0.21	3.92±0.05
CL	50	2.87±0.05	3.41±0.10	4.24±0.07	4.79±0.07	4.67±0.09

Values are means ± SEM (Standard Error Mean), n=5
Differences by one way ANOVA followed by Tukey HSD post hoc,
*p<0.05, **p<0.005, ***p<0.001 from respective standard i.e. Diclofenac Sodium.

Table 2: Analgesic Activity by Tail Flick Method (100 mg /kg)

Drug	Dose mg/kg	Time (Sec)				
		0	30	60	90	120
Control	0.3 ml	1.03±0.03	1.14±0.08	1.07±0.03	1.12±0.08	1.17±0.06
Standard	100	3.01±0.04	4.54±0.25	4.89±0.17	5.16±0.10	5.09±0.12
C.Q	100	2.73±0.09	3.5±0.14	3.68±0.09	3.87±0.12	4.12±0.09
L.S	100	2.48±0.03	3.12±0.04	3.76±0.08	4.10±0.11	4.18±0.08
CL	100	2.96±0.03	3.53±0.27	4.26±0.04	4.83±0.05	4.69±0.11

Values are means ± SEM (Standard Error Mean), n=5
Differences by one way ANOVA followed by Tukey HSD post hoc,
*p<0.05, **p<0.005, ***p<0.001 from respective standard i.e. Diclofenac Sodium.

Table 3: Analgesic Activity by Tail Flick Method (200 mg / kg)

Drug	Dose mg/kg	Time (Sec)				
		0	30	60	90	120
Control	0.3 ml	1.1±0.05	1.14±0.08	1.07±0.03	1.12±0.08	1.17±0.06
Standard	100	3.01±0.04	4.54±0.25	4.89±0.17	5.16±0.10	5.09±0.12
C.Q	200	2.71±0.03	3.44±0.11	3.62±0.06	3.92±0.08	4.02±0.13
L.S	200	2.53±0.04	3.17±0.20	3.61±0.12	3.81±0.06	3.78±0.05
CL	200	3.03±0.05	3.48±0.04	3.95±0.02	4.36±0.04	4.27±0.10

Values are means ± SEM (Standard Error Mean), n=5
Differences by one way ANOVA followed by Tukey HSD post hoc,
*p<0.05, **p<0.005, ***p<0.001 from respective standard i.e. Diclofenac Sodium.

Analgesic Activity by Hot Plate Method

Analgesic activity at dose of 50, 100 and 200mg/kg showed all extracts were found significant $p<0.05$ analgesic activity, in respective to negative control i.e. normal saline. Analgesic activity at dose of 50mg/kg showed significantly potent activity of CL at 120 min as compared to diclofenac sodium.

At dose of 100mg/kg showed significantly moderate activity of CL from respective standard i.e. Diclofenac Sodium. Whereas at dose of 200mg/kg showed significantly potent activity of LS and CL while significantly moderate activity of CQ from respective standard i.e. Diclofenac Sodium.

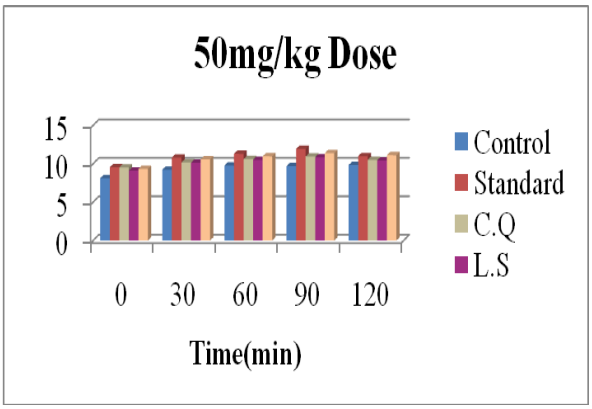

Fig. 3: Analgesic Activity by Hot Plate Method (50 mg / kg)

Values are means ± SEM (Standard Error Mean), n=5
Differences by one way ANOVA followed by Tukey HSD post hoc,
*p<0.05, **p<0.005, ***p<0.001 from respective standard i.e. Diclofenac Sodium.

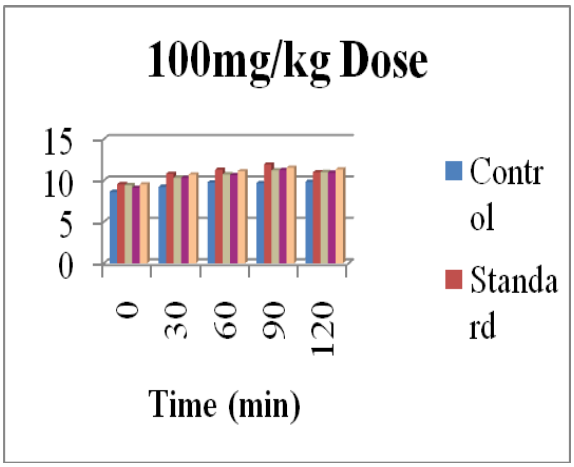

Fig. 4: Analgesic Activity by Hot Plate Method (100 mg / kg)

Values are means ± SEM (Standard Error Mean), n=5
Differences by one way ANOVA followed by Tukey HSD post hoc,
*p<0.05, **p<0.005, ***p<0.001 from respective standard i.e. Diclofenac Sodium.

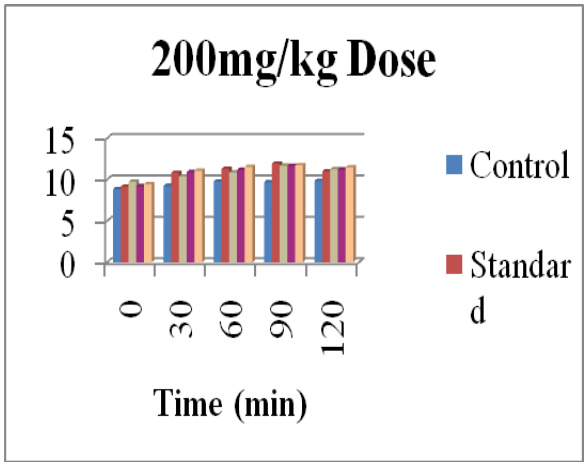

Fig. 5: Analgesic Activity by Hot Plate Method (200 mg / kg)

Values are means ± SEM (Standard Error Mean), n=5
Differences by one way ANOVA followed by Tukey HSD post hoc,
*p<0.05, **p<0.005, ***p<0.001 from respective standard i.e. Diclofenac Sodium.

DISCUSSIONS

The analgesic activity of both extracts and combination are significant as compare to normal saline while results of the combination extract was found as potent as diclofenac sodium at dose of 50 and 100 mg/kg. At 200mg/kg dose also showed significant analgesic activity by tail flick method. While by hot plate method significantly potent analgesic activity of CL was observed at 120 min at dose of 50mg/kg, also LS and CL at dose of 100mg/kg in comparison to diclofenac sodium.

The efficacy of the most herbal remedies is attributed to various active principles in combination. The antioxidant capacity of some plants is due to polyphenol metabolites. The most important of these polyphenol compounds are the flavonoids which are endogenous in the brain and appear to have neuroprotective effect and favorable bioavailability. Both extracts contain flavonoids principles are some of the widest spread poly phenolic compounds in the plant world and having a wide range of pharmacological effects. It is accepted that metabolic transformations i.e. glucoronidation and methylation of the flavonoids occur in the body and that a very small amount of flavonoids are feed as aglycons in the blood[27]. One of the important flavonoids is reservatral mainly while oxysterol occasionally found in these two plants. They are initially identified as defensive response against stress, UV radiation, pathogens and physical damage[28]. The primarily target of pain sensation in all these extracts are prostaglandins due to the presence of flavonoids which involved in controlling pain

perceptions[29,30]. The combination extract has the double potential of flavonoids which inhibiting or antagonizing the enzyme cyclooxygenase causes suppression of formation of prostaglandins.[31]

CONCLUSION

Antioxidant activity of these compounds in synergy when used in combination is responsible for analgesic activity and suppression of pain leading to boost the immune system especially reservatrol (3'4'5, trihydroxy stilbenes) is found miraculously more effective in protecting oxidative damage even when Vitamin E and C combined. As interestingly improved response of extracts observed when used in combination along with high level of Vitamin C contents present in plant extracts.

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