

Potential anti-inflammatory effect of *Cissus quadrangularis* L. and *Lepidium sativum* L. along with their combination extracts

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Abstract: The anti-inflammatory activity of methanolic root extract of *Cissus quadrangularis* L. (CQ) and seed extract of *Lepidium sativum* L. (LS) were studied in rats also in conjunction with using a mixture (CL) of CQ and LS. The estimation of anti-inflammatory activity was conducted by measuring volumetrically with the help of plethysmometer, the mean increase in hind paw volume of rat. Standard drug like diclofenac sodium within the dose of a 100 mg/kg is used. The doses of 50, 100 and 200 mg/kg of both plant extracts individually as well as combination were given. 0.9% NaCl (saline) solution given to control group. All the doses administered orally. Results showed that at dose of 50mg/kg showed potent activity by LS(4.06±0.03) and CQ(4.16±0.03), as CL (3.96±0.03) showed moderate activity while dose 100 and 200 mg / kg showed moderate activity by LS from respective standard i.e. Diclofenac Sodium.

Keywords: *Cissus quadrangularis* L (CQ), *Lepidium sativum* L (LS), combination extract (CL), anti-inflammatory, Paw volume, hind paw edema method.

INTRODUCTION

Many drugs at this era are put into practice for the treatment of pain and inflammatory diseases which can be both narcotics as well as non narcotics in nature. This is moreover found medically during research that these drugs have been established with high aspect and poisonous consequences. It is consequently essential to increase drug treatments from plant sources of inexpensive value, with low side effects than the synthetic drugs which are very expensive to formulate. In indigenous system of drugs, stems of *C. quadrangularis* and seeds of *L. sativum* were selected as anti-inflammatory drugs for their reported pharmacological activities.

Cissus quadrangularis Linn (CQ) is a medicinal herb alleged to be of valuable effect within the conventional system of medicine. The CQ is commonly referred as Hajoda (Fam. Vitaceae) is one of the most extensively used components in alternative medicine (Ayurveda) for piles, anorexia, indigestion, chronic ulcers, asthma, otorrhoea, wounds and in augmenting fracture healing process (Agarwal 1997).

The alcoholic extract of this plant has evaluated via Udupa *et al.* (1965) mentioned to facilitate remedial of fractured bones in albino rats via intramuscular administration. Phytochemical studies reveal the presence of renowned flavonols including quercetin and kaempferol along side resveratrol, piceatannol, pallidol, ascorbic acid, ketosteroid and carotene (Saburi, 1999; Sen, 1966). Flavonoids are some of the widest unfold

phenolic compounds within the plant kingdom and having a wide variety of pharmacological outcome.

The *Lepidium Sativum* L. (LS) is a native shrub. The *Lepidium Sativum* seeds contain volatile essential aromatic oils, active principle ami fatty oils and carbohydrate, protein, fatty acid, Vitamin: P-carotene, riboflavin, and niacin, and ascorbic acid, Flavonoids, Isothiocyanates glycoside (Udupa *et al.*, 1965). *Lepidium sativum* seeds are considers being a valuable medicinal remedy to cure anti-inflammatory and analgesic (Al-Yahya *et al.*, 1994). In rheumatic joints the paste of seeds is applied to relieve the pain and swelling (Ahsan *et al.*, 1989).

Traditionally both plants are used for anti-inflammatory and in the best of our knowledge, studies on the CQ with LS as combination on anti-inflammatory effect is not reported. On this basis, we investigated the activity of the methanolic extract of the CQ and LS as a combination on anti-inflammatory activity, in rat which can be worthwhile to clinical health.

MATERIALS AND METHODS

Collection and identification

Fresh plant stems of *Cissus quadrangularis* had been accumulated 3.0 kg from medicinal plants lawn Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi. The seeds of *Lepidium sativum* 2.0 kg was purchased by local herbal store, Karachi. Both the samples were identified and authenticated by the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi.

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Preparation of plant extract

3 kg Fresh plant CQ, washed below strolling tap water to remove sticking dust, into small portions herbs were chopped off and for thirty days soaked in methanol after which extracted using methanol two times, accompanied by way of filtration via Whatman no.2 filter paper, previous to evaporation. By the use of a rotary vacuum evaporator the filtrate became evaporated to dryness in getting extract (Iwaki, Tokyo).

For the seeds of LS (2.0kg) the same soaking procedure we perform into methanol for thirty days, then extracted using methanol two times, accompanied via filtration Whatman filter paper, a rotary vacuum evaporator used for evaporated to dryness in getting extract (Iwaki, Tokyo). In the ratio of 70:30, CQ and LS these two extracts are combined.

Animals

The pharmacological experiments were conducted by using Swiss albino rats weighing 160-200g of both sex and acute toxicity on mice. Throughout the test under standard nutritional and environmental situations animals have been maintained at 25 ± 5 °C temperature, $50 \pm 10\%$ RH and 12 h light and 12 h dark cycle.

After an acclimatization period of at the least 5 days the animals had been used to the laboratory environment and provided with standard meals pellets and water *ad libitum*.

Before experimentation for 24hours the animals were deprived of food. From the institution for the present study the animal ethical committee clearance was obtained.

Acute toxicity test

Mice were divided into groups of ten each and in doses from 50 to 2000 mg/kg extracts were injected i.p. Death within 24 h was recorded⁷.

Anti-inflammatory activity

Hind paw edema technique

In present study by method of Winters et al⁸ in albino rats of either sex anti-inflammatory activity was determined. Under the plantar aponeurosis anti-Inflammatory activities were measured against acetic acid induced in the right hind foot. The rats were divided into five groups of six each. Group first received normal saline (0.1 ml/10 g i. p.). Extract of CQ, LS and CL received by groups second, third, and fourth orally at doses of 50, 100 and 200 mg/kg, respectively, one hour before injection 1ml/100gm of 1% solution of acetic acid was given. Group five received Diclofenac sodium (100 mg/kg po.).

The inflammation was quantitated in terms of ml i.e. replacement of water by edema using a plethysmometer

(Ugo Basile) (Santos *et al.*, 2004), immediately before Acetic acid injection and then 1, 2 and 3, hours after Acetic acid injection. The percent inhibition of edema as calculated for each group with respect to its vehicle-treated control group. The anti-inflammatory activity was calculated by using the relation used by Planichamy (Palanichamy and Nagarajan 1990).

$A-B/A \times 100$

Where A mean increase in paw volume of control

B denote mean increase in paw volume of drug-treated animals respectively.

STATISTICAL ANALYSIS

Mean increase in paw volume \pm SEM (Standard Error Mean) were expressed as values for anti-inflammatory activity. Between means the significance of difference was determined by ANOVA values of $p < 0.05$ were considered significant and $p < 0.01$ as highly significant.

RESULTS

The acute toxicity activity conducted showed safe usage as all the extracts does not produce any toxic effect on oral administration in mice from dose 50 to 2000 mg/kg and no death had been observed within 24 h.

In methanolic extract the anti-inflammatory potential in albino rats has been determined by using hind paw edema assay. The anti-inflammatory effects of Diclofenac sodium (standard drug) and methanolic extracts of CQ, LS and CL are shown in tables 1-3 respectively. In terms of ml and percentage inhibition in paw volume by different doses of the extracts, the anti-inflammatory activity was expressed as "mean increase in paw volume \pm SEM". LS and CQ at dose of 50mg/kg showed potent activity and CL showed moderate activity while LS at dose 100 and 200 mg / kg showed moderate activity from respective standard i.e. Diclofenac Sodium.

DISCUSSION

In complementary medicines one of the most important aspects is herbal medicine. The best classical approaches in exploration of new lead molecules for management of different ailments, as herbs are evaluated on experimental animal models.

In present era inflammation is a very immense challenge of mankind. So much of anti-inflammatory drugs such as opioids and analgesia are available, but these drugs like NSAIDS are not useful in all cases and these drugs also produce side effects, so to conquer this problem new drugs are requisite. Herbal medicines have many phytoconstituents which are supportive and also curative in inflammation and have fewer side effects.

Table 1: Anti-inflammatory Activity by HIND PAW EDEMA METHOD (50 mg / kg)

Drug	Dose mg/kg	Time (hr) volume in mm			
		0	1	2	3
Control	0.3 ml	2.2±0.06	3.6±0.03	4.0±0.05**	4.1±0.05**
Standard	100	2.2±0.06	3.23±0.03*	3.53±0.03	3.73±0.06
C.Q	50	2.26±0.06	3.53±0.03	3.9±0.05*	4.06±0.03**
L.S	50	2.20±0.057	3.63±0.12*	4.06±0.08**	4.16±0.03***
CL	50	2.3±0.057	3.43±0.06	3.83±0.08	3.96±0.03*

**Potent activity= LS and CQ

* Moderate activity= CL from respective standard i.e. Diclofenac Sodium.

Table 2: Anti-inflammatory Activity by HIND PAW EDEMA METHOD (100 mg / kg).

Drug	Dose mg/kg	Time (hr)			
		0	1	2	3
Control	0.3 ml	2.23±0.06	3.6±0.03	4.0±0.05*	4.1±0.05*
Standard	100	2.23±0.06	3.23±0.03	3.53±0.03	3.7±0.06
C.Q	100	2.23±0.06	3.4±0.11	3.76±0.12	4.03±0.03
L.S	100	2.3±0.057	3.5±0.08	3.93±0.03*	4.13±0.12*
CL	100	2.23±0.12	3.3±0.16	3.76±0.12	3.96±0.08

* Moderate activity= LS from respective standard i.e. Diclofenac Sodium.

Table 3: Anti-inflammatory Activity by HIND PAW EDEMA METHOD (200 mg / kg).

Drug	Dose mg/kg	Time (hr)			
		0	1	2	3
Control	0.3 ml	2.23±0.06	3.6±0.03**	4.0±0.05**	4.1±0.05*
Standard	200	2.23±0.06	3.23±0.03	3.53±0.03	3.73±0.06
C.Q	200	2.13±0.08	3.36±0.08	3.63±0.03	3.8±0.0
L.S	200	2.2±0.057	3.53±0.03*	3.63±0.06	3.96±0.08
CL	200	2.1±0.057	3.16±0.06	3.6±0.057	3.7±0.08

* Moderate activity= LS from respective standard i.e. Diclofenac Sodium.

The literature available on *Cissus quadrangularis* and *Lepidium Sativum* depicted an interesting fact that though the plant is a popular remedy for a variety of ailments. Their phytochemical studies shows the presence of triterpenoides, flavones, saponins, and alkaloids in the extract CQ while LS shows carbohydrate, protein, fatty acid, Vitamin: carotene, riboflavin, niacin, ascorbic acid, Flavonoids, and Isothiocynates glycoside (Udupa *et al.*, 1965).

Seeds of LS were used as potent anti-inflammatory effect, Moreover anti-inflammatory activity of CQ in rats had been reported by (Saratikov *et al.*, 2005) but no work have been done to test the antinflammatory activity of these plants in combination, thus to confirm the anti-inflammatory activity of these plants pharmacological evaluation had been carried out in this study.

It has been explicate from literature that flavnoids and terpenoids are major anti-inflammatory agents which are present in both plants. In different inflammatory

conditions they either act as phospholipase inhibitors or reported as TNF- α inhibitors. Flavonoids biochemical investigations have also shown inhibiting both cyclooxygenase and lipoxygenase pathways of arachidonic metabolism depending upon their chemical structures (Chi *et al.*, 2001; Jang *et al.*, 2002). Terpenoids may affect different mechanism relevant to inflammations arising in response to varied etiological factors (Changa *et al.*, 2008).

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