International Standard Serial Number : 0976-1381

Deccan J. Natural Products 2(3): July-September 2011

DECCAN PHARMA JOURNAL SERIES

ARMS Online Publications

www.deccanpharmajournals.com

(Research Article)

Received; accepted

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF DIFFERENT EXTRACTS OF *OXALIS CORNICULATA* LINN

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Keywords:

Analgesic, anti-inflammatory activity, Petroleum ether extract, Ethyl acetate extract, Methanolic extract.

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ABSTRACT

The plant of *Oxalis corniculata* linn was traditionally used for the treatment of pains and inflammation. The present study was carried out using hot plate method and acetic acid-induced writhing tests in mice while, carrageenan-induced paw edema method in rats. The whole plant was subjected to successive extraction using the various solvents (petroleum ether, ethyl acetate, and methanol) in the increasing order of polarity. It was found that the *Oxalis corniculata* linn revealed the presence of alkaloids, steroids, flavonoids, terpenoids, glycosides, tannins and phenolic compounds. Both extracts (100 mg/kg, and 200 mg/kg) produced the significant ($P<0.01$) analgesic and anti-inflammatory effects. The observed pharmacological activities provide the scientific basis to support traditional claims as well as, exploring some new and promising leads.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicines. The plant-based traditional medicine system continues to play an essential role in health care with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary healthcare [1]. Pain is an unpleasant sensation no doubt; but pain is mainly a protective mechanism for the body. It occurs whenever any tissues damage or nerve damage or dysfunction in the peripheral or central nervous system, and it causes the individual to react to remove the pain stimulus. Analgesics are the drug that selectively relieves pain by acting in the central nervous system or on peripheral pain mechanisms, without significantly altering consciousness [2]. Inflammation is a localized reaction of the living body against an irritant (injurious agent) caused by release of chemicals from tissues and migrating cells, most strongly implicated are the prostaglandins, leukotrienes, histamine, bradykinin, and, more recently, interleukin-1 and platelet-activating factor in an attempt to destroy, dilute, or wall off that irritant [3]. Traditionally the plant is used in the treatment of influenza, fever, urinary tract infections, enteritis, diarrhea, traumatic injuries, sprains and poisonous snke bites. An infusion can be used as a wash to rid children of hookworms. The plant is good source of vitamin C and is used as an antiscorbutic in the treatment of scurvy. The leaves are used as an antidote poisoning by the seeds of Datura spp., arsenic and mercury. The leaf juice is applied to insect bites, burns and skin eruptions, it has an antibacterial activity. The plant is antihelmintic, analgesic, and anti-inflammatory, astringent, depurative, diuretic, emmenagogue, febrifuge, and lithontripic, stomachic and styptic.A decoction of leaves is used as a gargle [4]. Report on the antibacterial [5], antioxidant, antitumor [6], and antimicrobial [7], smooth muscle relaxant, cardio relaxant and hypotensive activity [8], antifungal activity [9], wound healing activity [10], nematocidal activity [11]. An extensive literature survey does not reveal analgesic and anti-inflammatory activity of whole plant. So the present study was undertaken to investigate the anti-inflammatory and analgesic activity of petroleum ether, ethyl acetate, and methanol extract of *Oxalis corniculata* linn.

2. MATERIAL AND METHODS

Plant Collection and Authentication

The fresh drug of *Oxalis corniculata* linn. were collected from Sangamner Dist.-Ahmednagar (Maharashtra); were authenticated by Dr.P.G.Diwakar. Joint director, Botanical survey of India, Pune.

Preparation of Extracts

After authentication fresh drug was collected in bulk, washed under

running tap water to remove adhering dust, dried under shade and powdered with the help of a mechanical grinder. The coarse powder was then used for extraction by using technique continuous hot Soxhlet extraction used different solvents in increasing order of polarity with petroleum ether (60-80^{0}c), ethyl acetate, and methanol. The extraction was carried out in Soxhlet extractor till all the constituents were extracted. (yield petroleum ether extract 13.41% w/w , ethyl acetate extract 9.85 % and methanolic extract 15.23 % with respect to dried material) [12].These different extracts thus obtained was then screened for analgesic and anti-inflammatory activity.

Experimental animals

Swiss albino mice (18-24 g) and Wistar rats (150–200 g) of either sex were procured from Shiram laboratories, Pune and were acclimatized for Institutional Animal Ethics Committee (Registration No. 1153/ac/07/CPCSEA). 5 days under standard housing condition maintained at a room temperature of 24 ± 1ºC; related humidity 45-55% with 12:12 hrs light/dark cycle. The animals were habituated to laboratory condition for 48 hrs prior to the experimental protocol to minimize any nonspecific stress.

Hot-plate test

The central analgesic activity of the test drug is studied against thermal stimuli using this method. The hot plate test was used to measure analgesic activity by the method described by Eddy and Leimback with minor modifications. In this test an electrically heated hot plate (Orchid Scientific Eddy's Hot Plate) was maintained at 55 ± 1^{0}C.The initial reaction time of all the animals of control and test groups were recorded by putting them on the hot plate maintained at 55±0.5^o C. Licking of paw or jumping was taken as the index of reaction to heat. The albino mice were divided into 8 groups, 6 mice in each group. Petroleum ether extract, ethyl acetate extract and methanolic extract of *O. corniculata* at a dose of (100 mg/kg and 200mg/kg body wt.each) and pentazocine injection (20 mg/kg body wt.) were administered by intra peritoneal route. The first group was served as control and received only vehicle (normal saline). Post treatment reaction time of each animal was recorded at 30, 60, 90, 120 and 180 min. The animals were removed from hot plate soon after they exhibited jumping. Cut off time was 20 seconds [13].

Acetic acid induced writhing

The painful stimulus is induced by i.p injection of an irritant substance (e.g. acetic acid) & peripheral analgesic activity is evaluated. In this method pain is generated indirectly via endogenous mediators like prostaglandin, which stimulates peripheral nociceptive neurons. Percentage protection against abdominal constriction was taken as an index of analgesia. The albino mice were divided into 8 groups, six mice in each group. *Oxalis corniculata* Linn

extract (100 mg/Kg and 200 mg/Kg body wt.each) and diclofenac sodium (20 mg/Kg body wt.) was administered one hour prior to intra peritoneal injection of 0.1 ml of 0.6% v/v acetic acid. Five minutes after the intra peritoneal injection of acetic acid, the number of writhing during the following 20 min. was counted. Control mice were received normal saline finally the percent analgesic effect was determined. The number of writhings and stretchings was recorded [14].

$$\text{Percentage of protection} = \frac{\text{Control mean - Treated mean}}{\text{Control mean}} \times 100$$

Anti-Inflammatory Activity:

Carrageenan Induced Paw edema:

Carrageenan is sulphated polysaccharide used in pain models which produces acute & chronic inflammatory responses. Albino rats of wistar strain of either sex were divided into eight groups. Acute inflammation was produced by sub plantar injection of 0.1ml of 1% suspension of carrageenan in normal saline, in the right hind paw of rats, one hour after oral administration of petroleum ether extract, ethyl acetate extract and methanolic extract (100 mg/kg and 200 mg/kg body wt.each) and the diclofenac sodium used as standard drug (20 mg/kg body wt.). Control rats were received only vehicle (normal saline). The paw volume was measured plethysmometrically (Medicaid Digital Volume Meter) at 0, 1, 2, 3, hours after the carrageenan injection. The difference between '0' readings and readings after 1, 2, and 3 hours respectively was taken as the volume of edema [15, 16]. The percentage inhibition of paw oedema in the various treated groups was then calculated by using the formula;

$$\text{Percentage inhibition} = \frac{1 - Vt}{Vc} \times 100$$

Where,

Vt = is the oedema volume in the drug treated group.

Vc = is the oedema volume in the control group.

Table 1: Effect of different extracts of *Oxalis corniculata* linn on hot plate method in mice.

Group	Latency to lick paw (sec)					
	0 min	30 min	60 min	90 min	120 min	180 min
Control	1.87±0.19	3.83±0.31	3.93±0.17	4.37±0.22	3.67±0.11	2.96±0.23
Pentazocine (20mg/kg)	1.48±0.24	3.21±0.19**	8.39±0.53**	14.75±1.68**	19.69±0.90**	13.23±0.70**
PEE (100mg/kg)	2.25±0.39	3.21±0.19*	8.26±0.57**	10.15±0.53**	14.06±0.57**	7.93±0.61*
PEE (200mg/kg)	2.45±0.30	3.65±0.10**	9.73±0.22**	16.3±1.13**	17.63±0.74**	7.38±0.52*
EAE (100mg/kg)	2.48±0.25	2.89±0.17	3.91±0.16	9.62±0.60	13.25±0.57*	11.83±1.10
EAE (200mg/kg)	2.2±0.31	3.19±0.82*	6.64±0.42*	11.59±0.46*	13.69±0.39*	9.50±0.10
MEE (100mg/kg)	2.82±0.15	2.85±0.15*	6.95±0.53*	11.82±1.11**	15.28±0.69*	8.62±1.85
MEE (200mg/kg)	2.78±0.24	3.65±0.35**	6.84±0.36**	13.29±1.15**	15.07±9.18**	10.24±2.03

n= Six, in each group. ** P < 0.01, *P < 0.05 compare with control, one way ANOVA followed by Dunnett's Test.

PEE= Petroleum ether extract, EAE= Ethyl acetate extract, MEE= Methanolic extract.

Table 2: Effect of different extracts of *Oxalis corniculata* linn on acetic acid induced writhing in mice.

Test Group	Mean ± S.E.M	% Inhibition
Control	22.8 ± 6.87	--
Diclofenac sodium (20mg/kg)	6.00 ± 1.73**	73.68 %
Petroleum ether extract (100mg/kg)	9.42±2.19**	58.68 %
Petroleum ether extract (200mg/kg)	6.60± 2.42**	71.05 %
Ethyl acetate extract (100mg/kg)	12.0 ± 3.42**	47.36 %
Ethyl acetate extract (200mg/kg)	7.89 ± 2.65**	65.39 %
Methanol extract (100mg/kg)	9.85 ± 1.83**	56.79 %
Methanol extract (200mg/kg)	6.63±0.3787**	70.92 %

n= six, in each group. ** P < 0.01, *P<0.05 compare with control, one way ANOVA followed by Dunnett's test.

Table 3: Effect of different extracts of *Oxalis corniculata* linn on carrageenan induced paw edema in rats.

Drugs	Difference in paw volume(in ml)mean± SEM				
	Reading at				
	0hr	1hr	2hr	3hr	4hr
Control	0.46 ± 0.01	0.53 ± 0.04**	0.69 ± 0.01*	0.73 ± 0.01**	0.76 ± 0.02**
Diclofenac 20 mg/kg	0.34 ± 0.05*	0.24 ± 0.03**	0.13 ± 0.01**	0.12 ± 0.01**	0.13 ± 0.01**
PEE 100 mg/kg	0.49 ± 0.41	0.5 ± 0.13*	0.416 ± 0.01*	0.42 ± 0.02**	0.41 ± 0.01**
PEE 200 mg/kg	0.47 ± 0.02	0.40 ± 0.04**	0.31 ± 0.01**	0.2 ± 0.02**	0.18 ± 0.01**

EAE 100 mg/kg	0.42 ± 0.47	0.45 ± 0.03*	0.38 ± 0.01*	0.36 ± 0.02**	0.36 ± 0.031*
EAE 200 mg/kg	0.43 ± 0.07	0.42 ± 0.02**	0.32 ± 0.01**	0.31 ± 0.03**	0.27 ± 0.12**
MEE 100 mg/kg	0.45 ± 0.09	0.47 ± 0.10*	0.4 ± 0.01**	0.40 ± 0.02**	0.42 ± 0.02**
MEE 200 mg/kg	0.46 ± 0.02	0.4 ± 0.04**	0.29 ± 0.01**	0.18 ± 0.01**	0.18 ± 0.01**

Values are mean ± S. E. M, *n=6,* $^{*}P<0.05$, significant as compared to control.

PEE= Petroleum ether extract, EAE= Ethyl acetate extract, MEE= Methanolic extract.

Tale 4: Percentage Inhibition at 3hr.

Group	**% Inhibition**
Control	-
Diclofenac sodium (20mg/kg)	83.56%
Petroleum ether extract (100mg/kg)	42.46%
Petroleum ether extract (200mg/kg)	72.60%
Ethyl acetate extract (100mg/kg)	50.68%
Ethyl acetate extract (200mg/kg)	57.53%
Methanolic extract (100mg/kg)	45.2%
Methanolic extract (200mg/kg)	75.34%

The effect of petroleum ether extract, ethyl acetate extract and methanolic extract of *Oxalis corniculata* Linn. at (100 mg/kg and 200 mg/kg) and pentazocin (20 mg/kg) was assessed by hot plate method shown in (Table 1). The results obtained from the Hot plate method indicated that petroleum ether extract had significant ($P < 0.01$) reduction in number of jumping or licking of the paw at the 60 minutes to 120 minutes time interval and persisted up to 180 min when compared to standard group. The ethyl acetate extract and methanol extract had significance reduction in number of jumping or licking of the paw ($P < 0.01$),at the 60 minutes to 90 minutes time interval and persisted up to 120 min when compared to standard group.

The peripheral analgesic activity of *O. corniculata* was determined in mice by acetic acid induced writhing method (Table 2). Pretreatment with pet-ether extract, ethyl acetate extract and methanolic extract of *O. corniculata* linn *at* the dose level of (100, mg/kg and 200mg/kg each) and standard diclofenac sodium significantly reduced the number of writhes. Petroleum ether extract had significant decreased in abdominal writhes to the extent of 71.05 %, when compared to standard group was 73.68 %. The ethyl acetate and methanol, extract was significance decreased in abdominal writhes, to the extent of 65.39 %, and 69% respectively, when compared to standard group 73%. Evalution of peripheral type of analgesic action there is significant decreased in writhing response induced by acetic acid in *O. corniculata* linn treated group, which proves its analgesic property The abdominal contraction is related to the sensitization of nociceptive receptors to prostaglandins. It is therefore possible that *O. corniculata* exerts its analgesic effect probably by inhibiting action of prostaglandins.

The results obtained from the carrageenan-induced rat paw edema indicated that petroleum ether extract, ethyl acetate extract and methanol extract of treated group showed ($P<0.01$) reduction in paw edema from 1^{st} to 4^{th} hr onwards when compared to standard group. Whereas diclofenac sodium also significantly ($P<0.01$) reduced paw edema form 1^{st} and 4^{th} hr. The result of anti-inflammatory activity was shown in (Table 3). The maximum inhibition in edema volume was noted (Table 4) to be at 3hr Pet ether extract 72.60%, Ethyl acetate extract 57.53% and methanol extract showed 75.34% comparable to the standard drug, Diclofenac sodium 20 mg/kg which caused maximum inhibition of 83.56% ($p < 0.05$).

CONCLUSION:

Based on the results of the present study it can be concluded that the methanolic extracts and petroleum ether extract of the *Oxalis corniculata* linn. has significant analgesic and anti-inflammatory activities. Hence this study has confirmed the use of the plant in traditional medicine as a treatment of pain and inflammation.

ACKNOWLEDGEMENT

We are especially thankful to Dr.M.J.Chavan to his valuable guidance and encouragement and providing the facilities to carry out the research work.

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