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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF EXTRACTS OBTAINED FROM *Oxalis corniculata* linn

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ABSTRACT

Oxalis corniculata linn is a plant that has traditionally been used to relieve pain and inflammation. In mice, the hot plate method and acetic acid-induced writhing tests were used, whereas in rats, the carrageenan-induced paw edema method was used. The entire plant was extracted in stages using several solvents (petroleum ether, ethyl acetate, and methanol) in sequence of increasing polarity. Alkaloids, steroids, flavonoids, terpenoids, glycosides, tannins, and phenolic substances were discovered in the Oxalis corniculata linn. The analgesic and anti-inflammatory effects of both extracts (100 mg/kg and 200 mg/kg) were significant (P0.01). The pharmacological activities discovered provide a solid foundation for both classic claims and the exploration of novel and potential leads.

Keyword(S): Analgesic, anti-inflammatory activity, Petroleum ether extract, Ethyl acetate extract, Methanolic extract

INTRODUCTION

For thousands of years, nature has provided medical substances, and an astounding number of modern medications have been identified from natural sources. Many of these discoveries were made based on how the agents were used in traditional medicine. Traditional plant-based medicine continues to play an important part in health care, with around 80% of the world's population relying on traditional medicines for their primary treatment **[1]**. Pain is an unpleasant sensation, but it is mostly a body's protection function. It occurs when tissues or nerves in the peripheral or central

nervous systems are damaged or dysfunctional, causing the individual to react in order to remove the pain stimulus. Analgesics are painkillers that work by acting on pain receptors in the central nervous system or on pain receptors in the peripheral nervous system without affecting consciousness **[2]**. Inflammation is a localised reaction of the living body to an irritant (injurious agent) caused by the release of chemicals from tissues and migrating cells, the most strongly implicated of which are 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]**. Flu, fever, urinary tract infections, enteritis, diarrhoea, traumatic injuries, sprains, and venomous snake bites have all been treated using the plant in the past.

Hookworms can be removed from children using an infusion as a wash. The plant is a good source of vitamin C and is used to treat scurvy as an antiscorbutic. The leaves are used to treat poisoning caused by Datura spp. seeds, arsenic, and mercury. The leaf juice contains antibacterial properties and is used to treat insect bites, burns, and skin outbreaks. The plant is astringent, depurative, diuretic, emmenagogue, febrifuge, lithontripic, stomachic, and styptic, as well as antihelmintic, analgesic, and anti-inflammatory. As a gargle, a decoction of leaves is utilized **[4]**. Antibacterial **[5]**, antioxidant **[6]**, anticancer, and antimicrobial activity **[7]**, smooth muscle relaxant, cardiac relaxant, and hypotensive activity **[8]**, antifungal activity **[9]**, wound healing activity **[10]**, and nematocidal activity are all reported **[11]**. An exhaustive literature search reveals that the whole plant has no analgesic or anti-inflammatory effect. The goal of this study was to see if petroleum ether, ethyl acetate, and methanol extracts of Oxalis corniculata linn have anti-inflammatory and analgesic properties.

MATERIAL AND METHODS

Plant Collection and Authentication

The fresh drug of *Oxalis corniculata* linn was collected from Sangamner Dist. Ahmednagar (Maharashtra); *and* was authenticated from Botanical survey of India, Pune.

Preparation of Extracts

Fresh medicine was collected in bulk after identification, rinsed under running tap water to remove adherent dust, dried in the shade, and powdered using a mechanical grinder. After that, the coarse powder was employed for extraction utilizing the continuous hot process. Petroleum ether (60-80^{0}C), ethyl acetate, and methanol were used in Soxhlet

extraction in sequence of increasing polarity. The extraction was completed in a Soxhlet extractor until all constituents had been removed. (relative to dried material, produce petroleum ether extract 13.41 % w/w, ethyl acetate extract 9.85 %, and Methanolic extract 15.23 %) **[12]**. The analgesic and anti-inflammatory efficacy of the various extracts obtained was then tested.

Experimental animals

Shriram laboratories in Pune provided Swiss albino mice (18-24 g) and Wistar rats (150–200 g) of either sex, which were acclimatized for the Institutional Animal Ethics Committee (Registration No. 1153/ac/07/CPCSEA). 5 days in typical living conditions with a room temperature of 24°C, relative humidity of 45-55 percent, and a light/dark cycle of 12:12 hours. To reduce nonspecific stress, the animals were acclimated to laboratory conditions for 48 hours prior to the experimental protocol.

Hot-plate test

This method is used to investigate the test drug's central analgesic action in response to temperature stimulation. The method provided by Eddy and Leimback with slight modifications was utilized to determine analgesic activity using the hot plate test. An electrically heated hot plate (Orchid Scientific Eddy's Hot Plate) was used in this experiment. All of the animals in the control and test groups had their initial reaction times recorded by placing them on a hot plate set to 55 ± 0.5^0C. The index of heat reactivity was licking of the paw or jumping. The albino mice were separated into eight groups, each with six mice. Pentazocine injection (20 mg/kg body wt.) and petroleum ether extract, ethyl acetate extract, and methanolic extract of *O. corniculata* at doses of (100 mg/kg and 200 mg/kg body wt.) were given intra peritoneal. The first group was designated as the control group and was given merely a car (normal saline). Each animal's post-treatment reaction time was measured at 30, 60, 90, 120, and 180 minutes. The animals were taken off the hot pan as soon as they started jumping. The time limit was 20 seconds **[13]**.

Acetic acid induced writhing

An irritating chemical (e.g. acetic acid) is injected intravenously to create a painful stimulus, and peripheral analgesic action is measured. Pain is produced indirectly in this technique by endogenous mediators such as prostaglandin, which excite peripheral nociceptive neurons. As an indicator of analgesia, the percentage of protection against abdominal constriction was used. The albino mice were separated into eight groups, each with six mice. One hour before intra peritoneal injection of 0.1

ml of 0.6 percent v/v acetic acid, Oxalis corniculata Linn extract (100 mg/Kg and 200 mg/Kg body wt.) and diclofenac sodium (20 mg/Kg body wt.) were given. The number of writhing for the next 20 minutes was counted five minutes after the intraperitoneal injection of acetic acid. The % analgesic effect was evaluated when control mice were given normal saline. A total of writings and stretching were counted **[14]**.

$$\textbf{Percentage of protection} = \frac{\textbf{Control mean} - \textbf{Treated mean}}{\textbf{Control mean}} \textbf{ x 100}$$

Anti-Inflammatory Activity:

Carrageenan Induced Paw edema:

Carrageenan is a sulphate polysaccharide that causes acute and chronic inflammatory reactions in pain models. Eight groups of albino Wistar rats of either sex were created. Acute inflammation was induced in rats by injecting 0.1ml of 1% carrageenan suspension in normal saline into the right hind paw 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 standard drug diclofenac sodium (20 mg/kg body wt.). Only vehicle was given to the control rats (normal saline). At 0, 1, 2, and 3 hours after the carrageenan injection, the paw volume was measured plethysmometrically (Medicaid Digital Volume Meter). The volume of edema was calculated as the difference between '0' values and readings after 1, 2, and 3 hours, respectively. Using the formula, the percentage inhibition of paw edema in the various treatment groups was computed **[15, 16]**.

$$\textbf{Percentage inhibition} = \frac{\textbf{1 - Vt}}{\textbf{Vc}} \textbf{ x 100}$$

Where

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

Vc = is the edema 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 hot plate method was used to evaluate the effects 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) **(Table 1)**. When compared to the control group, the results from the Hot plate method revealed that petroleum ether extract reduced the number of jumping or licking of the paw by a substantial (P 0.01) amount from 60 to 120 minutes and lasted up to 180 minutes. When compared to the control group, the ethyl acetate extract and methanol extract had a significant reduction in the number of jumping or licking of the paw (P 0.01) between 60 and 90 minutes, and this effect lasted up to 120 minutes.

The acetic acid induced writhing method was used to test the peripheral analgesic activity of *O. corniculata* in mice **(Table 2)**. The number of writhes was

dramatically reduced after pretreatment with pet-ether extract, ethyl acetate extract, and methanolic extract of *O. corniculata* linn at dose levels of (100, mg/kg, and 200mg/kg, respectively) and normal diclofenac sodium. When compared to the control group, which had a 73.68 percent reduction in abdominal writhes, petroleum ether extract had a significant reduction of 71.05 percent. When compared to the control group, which had 73 percent abdominal writhes, the ethyl acetate and methanol extracts significantly, reduced abdominal writhes by 65.39 percent and 69 percent, respectively. When the peripheral type of analgesic activity of *O. corniculata* linn is evaluated, the writhing reaction caused by acetic acid is significantly reduced, proving its analgesic nature. The sensitivity of nociceptive receptors to prostaglandins is linked to abdominal contraction. As a result, it's probable that *O. corniculata* works as an analgesic by suppressing the function of prostaglandins.

When compared to the control group, the results from the carrageenan-induced rat paw edema showed that the petroleum ether extract, ethyl acetate extract, and methanol extract of the treated group demonstrated (P0.01) reduction in paw edema from the 1st to 4th hr. Diclofenac sodium, on the other hand, significantly (P0.01) reduced paw edema from the first to the fourth hour. Anti-inflammatory action was demonstrated in **(Table 3)**. The largest reduction in edema volume was observed at 3 hours **(Table 4)**. Pet ether extracts showed 72.60 percent inhibition, ethyl acetate extract 57.53 percent inhibition, and methanol extract 75.34 percent inhibition, all of which were equivalent to the reference medicine Diclofenac sodium 20 mg/kg, which generated maximum inhibition of 83.56 percent (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.

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