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Original Article

Analgesic and Anti-inflammatory Properties of *Oxalis dehradunensis* Raizada in Rats

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ABSTRACT

Background: Inflammation is a complex biological response that plays a key role in the body's defence mechanisms against various injuries and infections. Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) are widely used to treat pain and inflammatory conditions, but their long-term use is associated with adverse effects. Therefore, there is a need to explore alternative therapies for pain and inflammation management. Plant extracts are an excellent source of natural compounds that possess anti-inflammatory and analgesic activity. **Objectives:** The study aims to investigate the anti-inflammatory and analgesic potential of ethanolic extract of *Oxalis dehradunensis* leaf powder. **Materials and Methods:** The extraction of the plant part was carried out using ethanol in Soxhlet apparatus. Preliminary phytochemical screening of the ethanolic extract was performed to check the phytoconstituents. In the *in vivo* study, inflammation was induced in albino Wistar rats by causing hind paw oedema using carrageenan. Hot plate and Tail flick model was used to evaluate analgesic activity. Diclofenac sodium was used as a standard for inflammatory and analgesic activity. **Results:** The findings suggest that ethanolic leaf extract of *Oxalis dehradunensis* showed the presence of flavonoids, carbohydrates, phenols and tannins. At doses of 200 and 400 mg/kg body weight, the ethanolic leaf extract of *Oxalis dehradunensis* exhibited significant anti-inflammatory activity against carrageenan-induced paw edema following injection. In analgesic activity (Hot plate and Tail flick model) the results showed that *Oxalis dehradunensis* Raizada extracts at doses of 200 and 400 mg/kg increased the reaction latency time in both tests, indicating a significant antinociceptive effect. **Conclusion:** The ethanolic leaves extract of *Oxalis dehradunensis* exhibited significant anti-inflammatory and analgesic effects.

Keywords: Analgesia, Flavonoids, Inflammation, *Oxalis dehradunensis*, Polyphenols.

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INTRODUCTION

Inflammation arises from tissue damage caused by infections (bacterial, viral, or fungal), physical agents, or immune system dysfunction. Its main aim is to neutralize harmful agents and subsequently clear damaged tissue to facilitate repair.[1] Neutrophils play crucial role by releasing various mediators that initiate, regulate, and ultimately resolve acute inflammation.[2,3] The resolution phase involves anti-inflammatory mediators and the recruitment of monocytes to remove cellular debris, crucial for restoring tissue homeostasis. When the body's natural processes fail to coordinate effectively, inflammation can become chronic, contributing to various diseases such as asthma, rheumatoid arthritis, inflammatory bowel disease, cancer, atherosclerosis, type 2 diabetes, obesity, and neurodegenerative disorders like Alzheimer's, Parkinson's, and multiple sclerosis.[4-6]

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The Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) used in the treatment of inflammation are among the most widely utilized classes of medications worldwide. However, their well-known undesirable side effects on the gastric mucosa, kidneys, bronchial system, and cardiovascular system have limited their use.[7] The current trend in research focuses on investigating plant-based medicines due to their affordability, accessibility, and minimal side effects.[8-10] In recent years, many researchers have concentrated on natural products derived from medicinal plants, such as flavonoids, steroids, polyphenols, coumarins, terpenes, stearic acid, and alkaloids, because of their broad pharmacological significance, including anti-inflammatory and analgesic properties, with fewer adverse effects.[11]

Oxalis dehradunensis Raizada, a plant commonly found in India, is frequently used in traditional

medicine to treat fever, pain, wounds, and inflammation. Known as Khatura, garden pink-sorrel, or *broadleaf woodsorrel*, this herbaceous perennial from the *Oxalidaceae* family is native to Mexico, South and Central America, and is widespread in the southeastern United States, Central America, the Caribbean,

and northern South America. The leaves of *Oxalis dehradunensis* Raizada contain tartaric acid, citric acid, calcium oxalate, flavones (such as apigenin), flavonols (such as quercetin), and glycoflavones (including vitexin and orientin), which contribute to its acidic taste due to the high oxalate content. *Oxalis dehradunensis* Raizada has traditional uses as an antiseptic, refrigerant, wound healer, anthelmintic, anti-inflammatory agent, astringent, diuretic, and in the treatment of urinary tract infections, fever, and cancer, as well as having antipyretic properties.[12-14] The present study was conducted to evaluate the anti-inflammatory and analgesic activities of *Oxalis dehradunensis* Raizada Ethanol Extract (ODREE), administered orally, in animal models of inflammation and pain to validate ethnopharmacological claims.

MATERIALS AND METHODS

Chemicals and Equipment

Acetic acid, hydrochloric acid, sodium carbonate, sodium chloride, sodium hydroxide, sodium phosphate were purchased from H.V. Technologies, Dehradun (India). Carrageenan, iodine, potassium iodide, potassium persulfate and sulfuric acid were obtained from Merck. Hot plates, mortar and pestles, conical flasks, measuring cylinders, syringes, scissors, electronic balances, oral gavage, test tubes, filtering funnels, filtering flasks, filter paper, and permanent markers were among the supplies and tools utilized in the experiments.

Collection and extraction of plant materials

In order to identify *Oxalis dehradunensis* Raizada, whole plant samples were obtained in the month of February 2024 from Dehradun. S. K. Singh of BSI, Dehradun, granted a certificate under reference number BSI/ NRC/ Herb (Ident.)/ 2024-25/56. Dried *Oxalis dehradunensis* Raizada leaf powder (50 g) was used for extraction with 300 mL of 95% ethanol as the solvent. This process was carried out using a Soxhlet apparatus, after which the solvent was evaporated using an electric water bath to concentrate the extract.

Phytochemical Screening

Using the Kalaiselvi *et al*. approach, the existence of flavonoids, alkaloids, carbohydrates, tannins, and phenolic was examined in extracts of *Oxalis dehradunensis* Raizada leaves.[15-17]

Animals and ethical aspects

Wistar albino rats (150-180 g) were used as experimental models, with six rats per group. They were given a 7-day acclimatization period to the laboratory environment and were provided with food and water ad libitum. The work protocol was approved by the Institutional Animal Ethics Committee (IAEC No. 264/ CPCSEA/IAEC/2024/07), and the study was conducted in the IAEC approved animal house of the School of Pharmaceutical Sciences, Shri Guru Ram Rai University, Dehradun, in 2024.

Anti-inflammatory activity

Carrageenan-induced paw oedema

The method was used to determine the anti-inflammatory activity of the extract. Animals were divided into four groups to evaluate the anti-inflammatory activity against acute inflammation. Group A (carrageenan control) did not receive any oral treatment. Group B (standard group) received 10 mg/kg of diclofenac sodium. Group C was administered 200 mg/kg of *Oxalis dehradunensis* Raizada, while Group D received 400 mg/kg of *Oxalis dehradunensis* Raizada, as the test plant extract. Inflammation was induced by injecting 0.1 mL of carrageenan (prepared as a 1% suspension in sterile normal saline) into the rats' left hind paws to cause edema. All treatments were given orally 1 hr prior to the carrageenan injection. Paw volume was measured using a plethysmometer before injection (0 hr) and at 2, 4 and 6 hr post-injection.[18-20]

The percentage inhibition was obtained as follows:

$$\%\ Inhibition = \frac{Increase\ in\ paw\ oedema\ (Control) - Increase\ in\ paw\ oedema\ (Test)}{Increase\ in\ paw\ oedema\ (Control)} \times 100$$

Analgesic activity

Hot plate method

Wistar albino rats of either sex (n=6), weighing 150-180 g, were used for the study. The analgesic properties of *Oxalis dehradunensis* Raizada were assessed using the hot plate method developed by Eddy and Leimbach. The temperature of the hot plate was maintained at 55±0.2°C, a level that is uncomfortable but does not cause skin burns. Animals displayed discomfort by licking or leaping.

The rats were administered the following treatments:

The control group received normal saline (0.9% v/w).

The test groups received oral doses of 200 mg/kg and 400 mg/kg of *Oxalis dehradunensis* Raizada extract.

The standard group received (10 mg/kg, i.p.) diclofenac sodium.

After receiving the medication or test material, the animals were tested at intervals of 0, 30, 60, and 120 min. The latency time, defined as the duration until the animal licked its front or rear paw or leaped off the plate, was recorded to assess analgesic

Table 1: Preliminary phytochemical screening of ethanolic extract of *Oxalis dehradunensis* leaves.

Contents	Ethanolic Extract
Alkaloids	Absent
Flavonoids	Present
Phenols and Tannins	Present
Carbohydrate	Present

Table 2: Effect of ethanolic leaves extract of *O. dehradunensis* carrageenan-induced hind paw oedema model of anti-inflammatory activity by plethysmometer All the values are in mL (Paw volume).

Extracts and compounds	Dose mg/kg	Reaction Time				Mean	% inhibition
		0 hr	2 hr	4 hr	6 hr		
Positive control+ Induction	1%v/w carrageenan	0.5±0.45	0.82±0.56	1.28±0.66	1.59±0.69	1.04±0.59*	(-)
Standard (Diclofenac)+ Induction	10 mg/kg	0.28±0.36*	0.35±0.28*	0.47±0.34**	0.27±0.65**	0.34±0.40**	67%
Ethanolic Extract + Induction	200 mg/kg	0.35±0.48	0.41±0.53*	0.59±0.57*	0.46±0.38*	0.45±0.49*	56%
Ethanolic Extract + Induction	400 mg/kg	0.29±0.56	0.36±0.51*	0.47±0.21**	0.28±0.3**	0.35±0.39**	66%

Values are mean ± S.E.M, *p<0.05 Versus control and Standard.

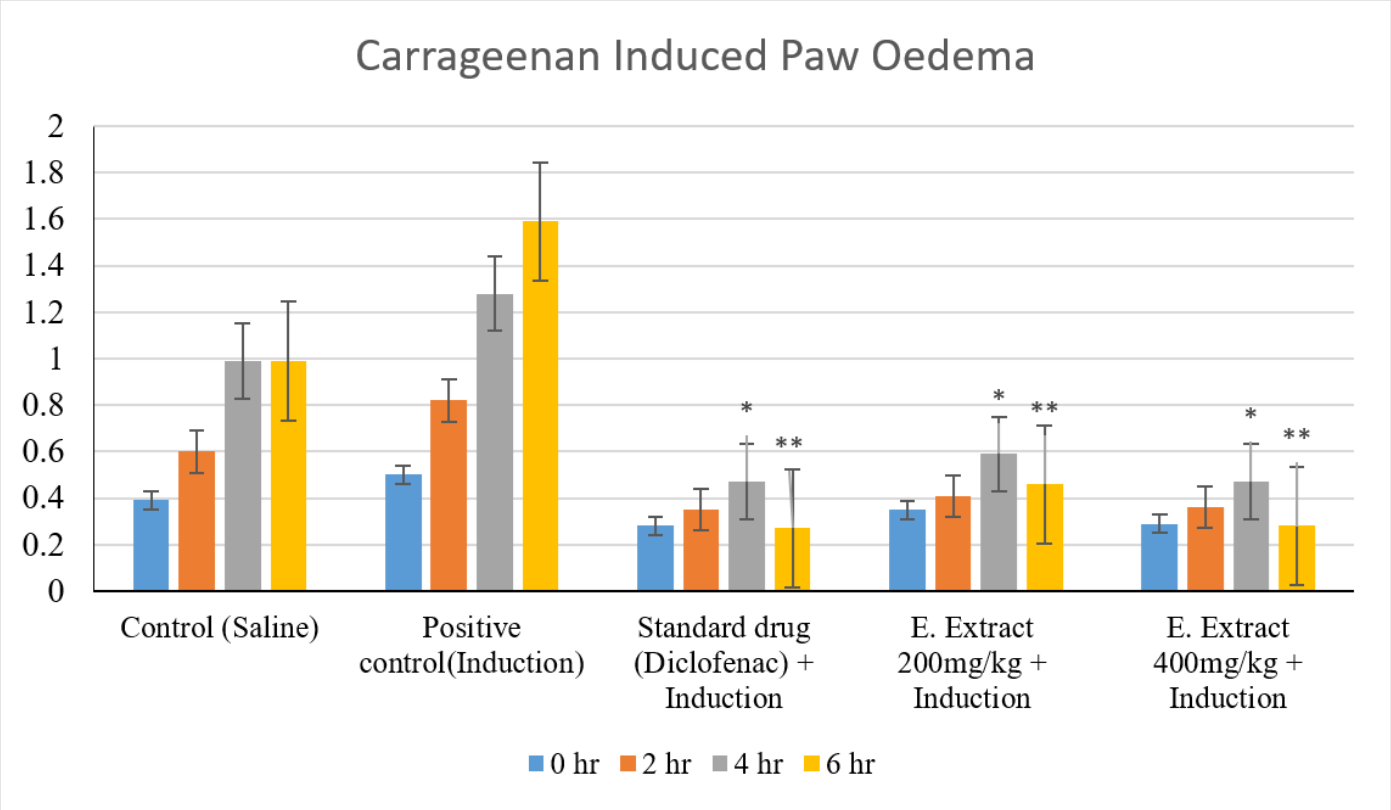

Figure 1: Graphical representation of effect of ethanolic extract (doses 200 and 400 mg/kg) of *O. dehradunensis* carrageenan induced hind paw oedema. Diclofenac sodium (10 mg/kg) was used as a standard drug. All values are expdressed as mean ± SEM.

response[21-23] 15 sec was used as post-treatment cut-off time. The percentage inhibition was obtained as follows:

$$\%\ Inhibition = \frac{post\ treatment\ latency - pre\ treatment\ latency}{Cut\ off\ time - pre\ treatment\ latency} \times 100$$

Tail flick method

Radiant heat was applied to a single spot on the proximal third of the tail using an analgesiometer. The time taken for the animal to withdraw (flick) its tail was recorded as the reaction time. The standard drug or test substance was administered after measuring the baseline reaction times at intervals of 0, 30, 60, and 120 min.[24,25]

Statistical Analysis

The results were analyzed using the statistical program GraphPad, with one-way ANOVA employed for group comparisons. A

Table 3: **Effect of *O. dehradunensis* by hot plate test, all the values are in second.**

Extracts and compounds	Dose mg/kg	Reaction Time			
		0 min	30 min	60 min	120 min
Control (saline)	10 mg/kg	4.44±0.35	6.0±0.46	4.65±0.28	5.61±0.52
Standard drug (Diclofenac)	0.9%v/w	4.44±0.35	6.63±0.36	7.82±0.20**	13.38±0.41**
Ethanolic Extract	200 mg/kg	1.82±0.23	3.77±0.49*	5.17±0.22*	8.41±0.43**
Ethanolic Extract	400 mg/kg	1.53±0.07	5.14±0.16**	6.65±0.49*	11.72±0.58**

Values are mean ± S.E.M. *$p < 0.05$ versus control, **$p < 0.01$ versus control.

Table 4: **Effect of *O. dehradunensis* by tail-flick test, all the values are in second.**

Extracts and compounds	Dose mg/kg	Reaction Time			
		0 min	30 min	60 min	120 min
Control (saline)	10 mg/kg	1.48±0.07	2.09±0.22	2.95±0.20	2.13±0.34
Standard drug (Diclofenac)	0.9% v/w	2.53± 0.37	5.0±0.22**	5.99±0.38**	5.73±0.42**
Ethanolic Extract	200 mg/kg	0.83±0.34	2.39±0.08*	4.29±0.05*	5.25±0.31*
Ethanolic Extract	400 mg/kg	0.97±0.23	4.32±0.14**	5.18±0.13**	5.53±0.02**

Values are mean ± S.E.M, *$p<0.05$ Versus control and Standard.

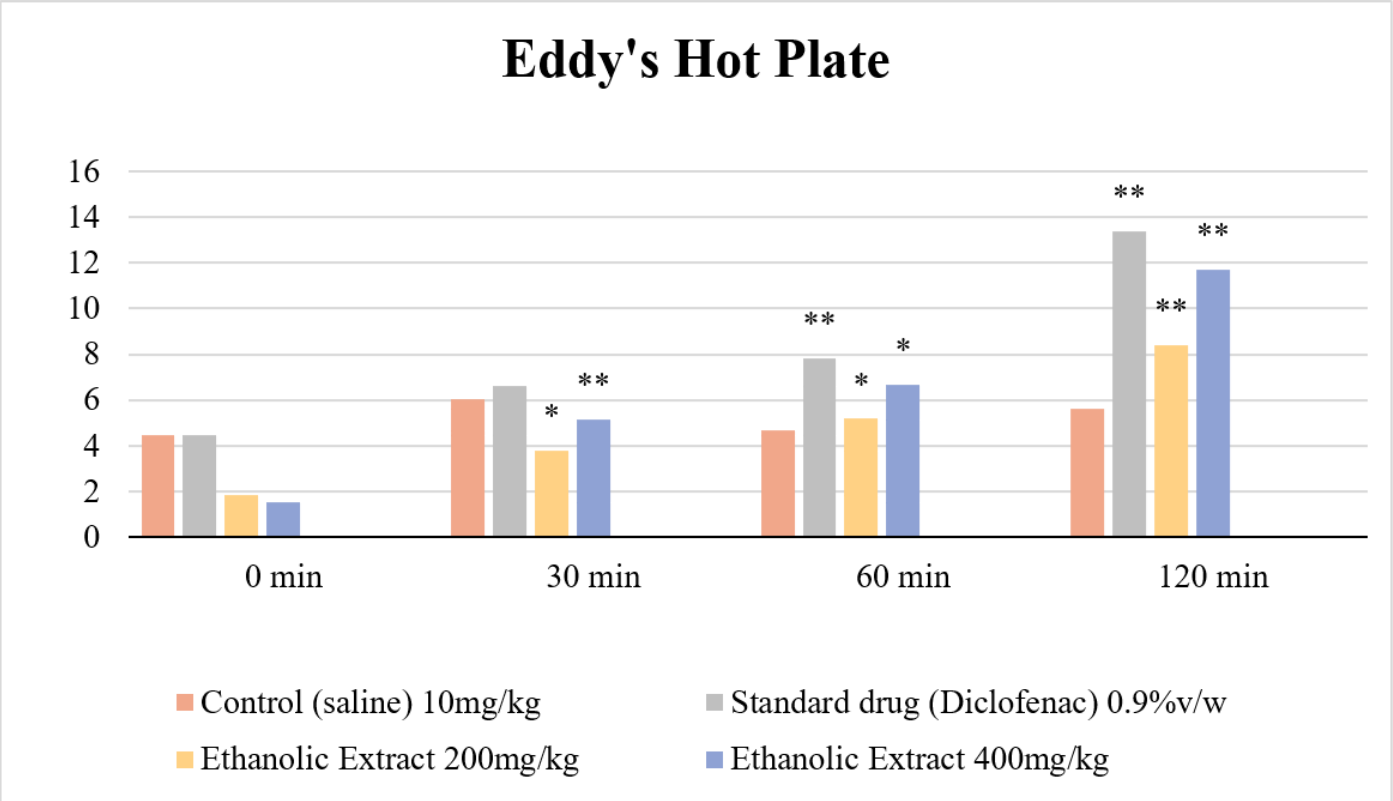

Figure 2: Graph Representation of effect of ethanolic extract (doses 200,400 mg/kg) of *O. dehradunensis* using Hot Plate. Diclofenac (10 mg/kg) used as a standard. All values are expressed as mean± SEM.

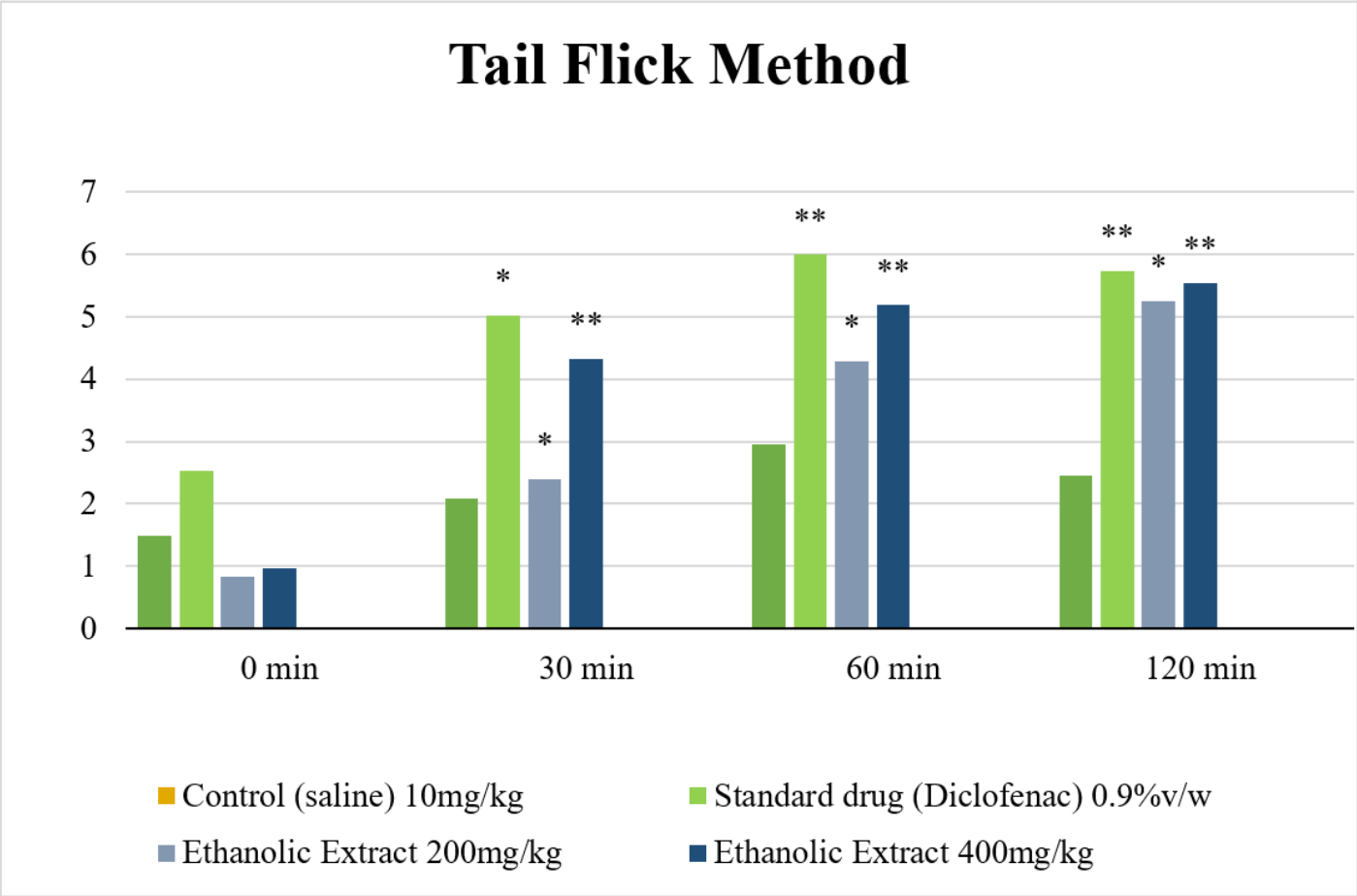

Figure 3: Graph Representation of effect of ethanolic extract (doses 200, 400 mg/kg) of *O. dehradunensis* using Tail Flick Method. Diclofenac (10 mg/kg) used as a standard. All values are expressed as mean± SEM.

p-value of less than 0.05 ($p<0.05$) was considered statistically significant. Data are presented as mean±S.E.M. (Standard Error of the Mean).

RESULTS

Phytochemical Screening

In extracts from *Oxalis dehradunensis* Raizada leaf, a variety of metabolites were discovered (Table 1).

Preliminary phytochemical study of the ethanol extract of *Oxalis dehradunensis* Raizada leaf, showed the presence of carbohydrates, phenols, tannins and flavonoids. The ethanol extract contained flavonoids, they belong to a class of components will protect from allergies, carcinogens and other harmful toxic substances.[26]

Anti-inflammatory activity-Carrageenan-induced paw oedema

The anti-inflammatory effects of the ethanol extracts of *Oxalis dehradunensis* Raizada on carrageenan-induced oedema in rat's hind paws are presented in Table 2 and Figure 1. The edema developed rapidly and was visibly apparent. It was observed that the edema peaked approximately 6 hr after Carrageenan induction in the control group. The extract demonstrated a dose-dependent reduction in edema. The 400 mg/kg dosage significantly reduced edema compared to the 200 mg/kg dosage and was comparable to the standard diclofenac sodium treatment.

Hot plate test

The results of the analgesic activity of the ethanolic extract of *Oxalis dehradunensis* Raizada are presented in Table 3 and Figure 2. Throughout the 120 min observation period, Wistar albino rats treated with normal saline (control) did not show any significant changes in their reaction times in Eddy's hot plate test. However, a significant improvement in reaction times was observed after administering two different doses of the *Oxalis dehradunensis* Raizada leaf ethanol extract. Animals treated with diclofenac sodium and *Oxalis dehradunensis* Raizada extract had significantly longer reaction times compared to the saline-treated group. At the 120-min mark, the reaction time for diclofenac sodium was 13.38 sec, 5.61 sec for the saline group, 8.41 sec for the *Oxalis dehradunensis* Raizada (200 mg/kg) group, and 11.72 sec for the *Oxalis dehradunensis* Raizada (400 mg/kg) group. The percentage inhibition of *Oxalis dehradunensis* Raizada (200 mg/kg) group, *Oxalis dehradunensis* Raizada (400 mg/kg) group, and diclofenac sodium, respectively, was 50%, 75.64% and 84.65% at 120 min. The increase in reaction time for diclofenac sodium also

varied significantly at different time points compared to baseline values within the same treatment group.

Tail flick method

Table 4 and Figure 3 presents the results of the ethanolic extract of *Oxalis dehradunensis* Raizada evaluated using the tail flick method. Wistar albino rats administered normal saline (control) exhibited no significant changes in their tail flick reaction times throughout the 120 min observation period. However, a marked improvement in reaction times was observed after administering two different doses of the *Oxalis dehradunensis* Raizada leaf ethanol extract. Reaction times were significantly longer in animals treated with diclofenac sodium and *Oxalis dehradunensis* Raizada extract compared to those treated with saline. At the 120 min mark, the reaction time for diclofenac sodium was 5.73 sec, while it was 2.13 sec for the saline group, 5.25 sec for the *Oxalis dehradunensis* Raizada (200 mg/kg) group, and 5.53 sec for the *Oxalis dehradunensis* Raizada (400 mg/kg) group.

DISCUSSION

The bioactive compounds present in herbs aid in treating and preventing various illnesses, boosting the body's defense mechanisms against infections, and promoting overall health.[27] The aim of the current study was to determine whether *Oxalis dehradunensis* Raizada extract possesses any antinociceptive or anti-inflammatory properties.

The anti-inflammatory properties of *Oxalis dehradunensis* Raizada extract were evaluated using a rat model of carrageenan-induced paw edema. The results demonstrated that *Oxalis dehradunensis* Raizada extract significantly inhibited edema, with the highest inhibition observed at 400 mg/kg of the extract. The inflammatory response, measured by paw swelling, was reduced following a single carrageenan injection. According to numerous studies, carrageenan-induced paw edema occurs in two stages: Stage I (0-2 hr after stimulation), associated with the release of histamine and 5-HT, and Stage II (2-6 hr after stimulation), which involves the production of inflammatory mediators such as bradykinin, COX-2, and prostaglandins.[28] Therefore, it is believed that *Oxalis dehradunensis* Raizada extract prevents carrageenan-induced inflammation by inhibiting cyclooxygenases, histamine, and 5-HT, which subsequently reduces prostaglandin formation.

The complex process of pain is mediated by numerous physiological mediators, including prostaglandins, substance P, bradykinins, and others. The current study used the hot plate and tail flick tests to investigate the antinociceptive properties of *Oxalis dehradunensis* Raizada extracts. The results showed that *Oxalis dehradunensis* Raizada extracts at doses of 200 and 400 mg/kg increased the reaction latency time in both tests, indicating a significant antinociceptive effect.

CONCLUSION

In conclusion, the ethanolic extract of *Oxalis dehradunensis* Raizada possesses analgesic and anti-inflammatory properties, providing a scientific basis for its ethnobotanical use in alleviating pain and inflammatory conditions. Additionally, a comprehensive study is currently underway to identify the active compounds responsible for these observed effects and to quantify pro and anti-inflammatory mediators, which will further elucidate the underlying mechanisms.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

Animal Ethics committee approval has been taken to carry out this study.

ABBREVIATIONS

NSAID: Non-Steroidal Anti-Inflammatory Drugs; **ODREE:** *Oxalis dehradunensis* Raizada Ethanol Extract; **COX-2:** Cyclooxygenase-2; **5-HT:** 5-Hydroxytryptamine; **IAEC:** Institutional Animal Ethics Committee; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **BSI:** Botanical Survey of India; **NRC:** National Referral Centre; **ANOVA:** Analysis of Variance; **SEM:** Standard Error of the Mean.

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