Studies on anti-inflammatory activities of whole plant of *Dendrobium macraei* Lindl.

Esha Vatsa[1*], Dr Samriti Faujdar[1], Shilpa Chandel[2], Nidhi Chaudhary[3], Ashok Kumar[2] Neeru[4]

[1*]Research Scholar Department of Pharmacy, Banasthali University, Rajasthan, India
[1] Department of Pharmacy, Banasthali University, Rajasthan, India
[2] Faculty of Pharmaceutical Sciences, The ICFAI University, Baddi, Himachal Pradesh, India
[3] Faculty of Pharmaceutical Sciences, Himgiri Zee University, Dehradun Uttarakhand, India
[4] Department of Pharmacy, Himachal Institute of Pharmacy, Ponta Sahib, Himachal Pradesh, India

ABSTRACT

The present study was carried out to evaluate anti-inflammatory activities in *Dendrobium macraei* whole plant extracts. Whole plant parts were extracted with Petroleum ether, chloroform, ethyl acetate, methanol and distilled water as the escalating order of polarity. The carrageenan induced rat paw edema model evaluated the anti-inflammatory activity of hydro-methanolic extracts. At doses 100, 200 & 400 mg/kg *D. macraei* exhibited significant and dose-dependent effects of anti-inflammatory activity. The current study's findings validated the folkloric use of *Dendrobium macraei* as an anti-inflammatory. The extracts exhibited a dose-dependent reduction in paw edema volume at different doses of 100, 200 and 400 mg/kg. The methanolic hydro extract at doses 100, 200 and 400 mg/kg significantly inhibited carrageenan-induced paw edema volume at 1h, 2h and most significantly at 3h as compared to the control. The standard drug indomethacin shows marked and significant inhibition in the volume of paw edema at 3hr at the dosage of 5 mg/kg through the oral route of drug administration.

Keywords: Anti-inflammatory, *Dendrobium macraei*, carrageenan-induced rat paw edema model.

Introduction

The International Association for the Study of Pain (IASP) defines pain as an unpleasant sensory and emotional experience connected with existing or potential tissue damage or defined in terms of such damage. Inflammation is a most exciting topic these days, as new research shows that inflammation triggers a chain of events that leads to most chronic diseases, including cancer[1].

Inflammation is the body's protective strategy for removing unwanted stimuli like germs, damaged cells, or irritants and initiating healing. Acute inflammation is the body's immediate response to a tissue injury produced by damaging stimuli, which occurs within seconds to minutes. If harmful foreign chemicals stay for an extended period and are not eliminated by the body, acute inflammation will become chronic[2].

NSAIDs (Nonsteroidal anti-inflammatory medicines) are commonly used to treat inflammation. Anti-inflammatory drugs reduce tissue damage and increase patient comfort by interfering with the pathophysiology of inflammation. The primary mechanism of action of NSAIDs is the central and peripheral inhibition of COX, which prevents arachidonic acid from being converted to prostaglandins E2, prostacyclins, and thromboxanes. Two enzymes that function in different places are involved in how NSAIDs work: COX-1 and COX-2. Most cells, including those in the fetal and amniotic fluid, contain COX-1, which is involved in physiological processes like regulation and defense. On the other hand, COX-2 is activated by cytokines that promote inflammation [3,4.]

But while opioids, used as potent analgesics, have harmful side effects, including addiction and dependence, they also have undesirable side effects like GI irritation, ulceration, and bleeding. As a result, scientists are becoming more interested in herbal medications because they may be safer and more efficient than NSAIDs and conventional analgesics. Natural compounds, which have lately been shown to be effective in preventing inflammation, powerfully scavenge inflammatory inducers. Many herbs, such as *Artemisia annua* and *Phaseolus vulgaris*, can help to reduce inflammation in a multi-faceted and balanced approach without the adverse side effects that medications can cause. Flavonoids, sterols, polyphenols, alkaloids, tannins, and terpenes are plant-derived natural compounds with a broad spectrum of pharmacological effects. The discovery of natural products with fewer adverse effects and less addictive potential, such as opioids, could be crucial in treating pain disorders and inflammation[5,6].

The World Health Organization (WHO) estimates that 65% of people worldwide receive medical care using traditional medicine (ethnobotanical applications). Herbal remedies are advancing topics in medicine, and we must learn more about them. Herbal prescription recommendations come mostly from complementary, alternative, and traditional medicines; however, contemporary medicine must confirm these principles through scientific means before employing them in practice[6,7].

Several trace elements in medicinal plants were found that play a crucial role in synthesizing active primary and secondary metabolites and provide therapeutic actions. Still, its abundance beyond allowed limits can lead to various toxicities[8].

From ancient times the genus *Dendrobium* has been used as the traditional drug for treating several diseases like Bowel diseases, impotence, asthma, bronchitis, general pain, inflammation, treatment of tuberculosis, a tonic for general debilities, general stimulant and emaciation also3. Based on an exhaustive literature survey, several chemical investigations on this plant exhibited the presence of alkaloids, flavonoids, carbohydrates, coumarins, phytosterols and phenolic compounds in the roots of *Dendrobium macraei*[9,10,11]. It is conspicuous here to facilitate the chemical above constituents that have been reported to acquire antidepressant, anti-inflammatory, and antimicrobial properties and are also used for managing diabetes. The genus Dendrobium has been investigated for anti-inflammatory activities[12]. However, no published data has yet been found concerning the anti-inflammatory activity of *Dendrobium macraei*. Based on the evidence above, the current study was planned to scientifically validate the anti-inflammatory activities of hydro-methanolic extracts of the whole plant of *Dendrobium macraei* scientifically.

Material and Methods

Collection and authentication of plant material

D. macraei's entire plant was harvested in Kankhal, Uttarakhand. The National Institute of Science Communication and Information Resources (NISCAIR), Delhi, Herbarium validated and identified the plant. The entire plant was then further shade-dried, ground into a coarse powder, and then sealed in a jar for later use.

Drugs, chemicals and instruments

Carrageenan suspension (PG Microlab Solutions, LLP, New Delhi, India), NaCl (Labogens, Gujarat, India), and indomethacin were the medications and substances employed in the current study (Chemimpex International, Kolkata, India). Analytical-grade materials were employed for all other compounds and reagents in the study. Model No. 166 UGO BASILE digital plethysmometer was utilized. We bought a rotary evaporator from Heidolph in Schwabach, Germany.

Extraction of plant material

The dried plant material was ground into powder using a mechanical grinder. The powdered material was then put through a series of Soxhlet extraction steps using a variety of solvents, including petroleum ether, chloroform, ethyl acetate, methanol, and distilled water, in increasing order of polarity. The powdered medicinal material was treated to drying in a hot-air oven below 50°C temperature before each Soxhlet extraction operation. The marc or residue was finally exposed to a 4-hour maceration with distilled water to prepare the aqueous extract. All extracts were then concentrated at a temperature of roughly 40°C in a rotating vacuum evaporator, after which the concentrated mixture underwent a freeze-drying procedure before being kept at 4°C for future use. After weighing each prepared extract, the percentage yield was determined using the weight of the relevant plant material after it had been air-dried[13,14]. Subsequent hydro methanol extracts of the entire plant were examined for their capacity to reduce inflammation.

Phytochemical Screening

The preliminary phytochemical screening of all the prepared extracts was conceded for detecting and evaluating alkaloids, proteins, amino acids, phenolic compounds, carbohydrates, glycosides, flavonoids and fats/oils, etc[15].

Animals

Wistar albino rats (*Rattus norvegicus*) weighing 150-200 gm were kept in the animal house of IDMA Laboratories Ltd, Panchkula, India, for experimentation. All animals were placed safely in clean polypropylene cages, and standard temperature conditions (22 ± 1℃) and simultaneous cycles of 12:12 hr light and dark were also maintained. Animals were provided with a proper diet (Hindustan Lever Ltd, India) of standard pellets and subjected to free exposure to water too. The Institutional Animal Ethical Committee genuinely approved all the procedures and protocols of the experiment used in the study (IAEC with Ref No. IAEC0122_04) and agreed with the guidelines of the CPCSEA.

Acute toxicity assay

An assay of acute toxicity was performed according to OECD guidelines 420. Wistar albino rats were randomly selected and separated into groups consisting of five animals per group. A

single dose of every test sample extract (2000 mg/kg) was administered orally to their assigned groups. The control group received the vehicle through the oral route. The rats were observed incessantly for the first 4 h and then periodically up to 24 h for toxic symptoms and mortality, if any.

Anti-inflammatory activity

Rat paw edema produced by carrageenan

Animals were divided into six groups using this procedure, with five in each group. The positive control was then allocated to Group No. I, did not receive any medicine. Group No. II has served as a negative control group and received carrageenan suspension (1%w/v) in 0.9% NaCl solution (0.1ml) by which acute inflammation was produced in male Wistar rats by injecting the prepared solution into the sub-plantar region of the rat's paw. Group No.III served as the standard group and was subjected to different doses of *Dendrobium macraei* plant extracts (100mg, 200mg and 400 mg/kg body weight, oral. route). Further, the difference between paw volumes of various groups was calculated and compared16. Paw volume was measured with a digital plethysmometer at intervals of 1, 2 and 3 h after carrageenan injection.

Statistical analysis

Data was depicted as mean ± S.E.M. for anti-inflammatory activity estimation. Statistical analysis for pharmacological activity was conceded by one-way ANOVA followed by the Dunnett test using Graph-Pad Prism software version 5.03. p-value was regarded as significant when $p < 0.05$.

Results

Phytochemical screening

It was found that the whole plant material of *Dendrobium macraei* contained carbohydrates, fats/oils, alkaloids, flavonoids, tannins and phenolic compounds. It is shown in Table No.1.

Table No. 1: Phytochemical screening of different extracts of *D*endrobium *macraei*

S.No.	Phytochemical tests	Petroleum ether Extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
1.	Carbohydrates	-	-	-	+	+
2	Fats & Oils	++	-	-	-	-
3.	Proteins	-	-	-	-	-
4.	Amino acids	-	-	-	-	-
5.	Alkaloids					
	Draggendroff's reagent	-	+++	++	-	-
	Mayer's reagent	-	++	-	-	-
	Hager's reagent	-	+	-	-	-
	Wagner's reagent	-	+	-	-	-
6.	Glycosides	-	-	-	-	-
7.	Flavonoids	-	-	+++	+	+

8.	Tannins and Phenolic compounds					
	Gelatin	-	-	+	+	+
	Bromine water	-	-	-	++	++
	Acetic acid solution	-	-	-	+	+
	Potassium dichromate	-	-	-	+	++

Note: (+) means positive, (-) means negative.

Anti-inflammatory activity

Rat paw edema produced by carrageenan

The anti-inflammatory activity responses of all *prepared D. macraei* whole plant extracts. All the extracts showed a dose-dependent reduction in paw edema volume at varied doses of 100, 200, and 400 mg/kg body weight. The hydro-methanolic extracts at variable doses, i.e., 100, 200 and 400 mg/kg, significantly ($p < 0.001$**)** inhibited the volume of carrageenan-induced rat paw edema at 2 h and most at 3 h as compared to the Negative control group. The standard indomethacin showed marked significant ($p < 0.001$) inhibition of paw edema volume at 3 h at the oral route dose of 5 mg/kg.

After 0 mint drug reaction

Normal Control	**CR**	**CR + Indo 5**	**CR + DM-100**	**CR + DM-200**	**CR + DM-400**
0.35	**0.34**	**0.34**	**0.34**	**0.34**	**0.35**
0.01	0.01	0.01	0.01	0.01	0.01
0.003	0.003	0.005	0.005	0.005	0.003

After 30 mint drug reaction

Normal Control	**CR**	**CR + Indo 5**	**CR + DM-100**	**CR + DM-200**	**CR + DM-400**
0.35	**0.66**	**0.37**	**0.56**	**0.53**	**0.49**
0.01	0.01	0.01	0.01	0.02	0.01
0.004	0.006	0.006	0.005	0.007	0.005

After 60 mint drug reaction

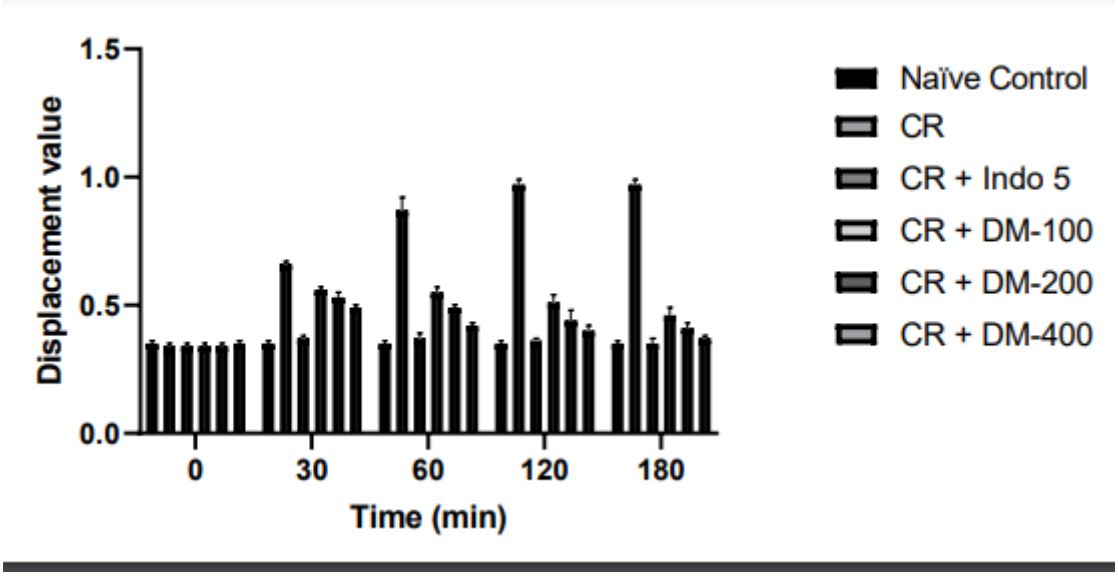
Normal Control	CR	CR + Indo 5	CR + DM-100	CR + DM-200	CR + DM-400
0.35	**0.87**	**0.37**	**0.55**	**0.49**	**0.42**
0.01	0.05	0.02	0.02	0.01	0.01
0.004	0.019	0.007	0.007	0.004	0.006

After 120 mint drug reaction

Normal Control	CR	CR + Indo 5	CR + DM-100	CR + DM-200	CR + DM-400
0.35	**0.97**	**0.36**	**0.51**	**0.44**	**0.40**
0.01	0.02	0.01	0.03	0.04	0.02
0.004	0.008	0.006	0.011	0.015	0.007

After 180 mint drug reaction

Normal Control	CR	CR + Indo 5	CR + DM-100	CR + DM-200	CR + DM-400
0.35	**0.97**	**0.35**	**0.46**	**0.41**	**0.37**
0.01	0.02	0.02	0.03	0.02	0.01
0.004	0.008	0.009	0.012	0.009	0.006



Discussion

According to a thorough examination of the literature, *D. macraei* has been used as a folk remedy for treating various conditions, including inflammation and general pain. The current study was designed to evaluate the claims based on historical uses of *D. macraei* for the uses

above. The preliminary phytochemical screening of *D. macraei* whole plant depicted the presence of alkaloids, carbohydrates, flavonoids, coumarins, phytosterols and phenolic compounds in roots. Traditionally, the species of *D. macraei* is a significant source for treating several ailments as it is derived from ayurvedic drug Jivanti which constitutes an alkaloid Jebantine and can be utilized as a stimulant and tonic17. Based on the literature survey, the plant is reported to constitute resinous principles α& β jibantic acids as chief phytoconstituents mainly present in stems and roots of plant18. Diosgenin derivatives like denfigenin and defuscin as steroids are also present in *D.macraei*19.The results developed from the literature surveydepicted that genus *Dendrobium* and its associated species also possess anti-platelet aggregation, anti-fibrotic, free radical scavenging, immunomodulatory, anti-inflammatory and cytotoxic activities and many more. No data was found based on the literature survey regarding the anti-inflammatory activity of this plant20. The results of the present investigation showed that the carrageenan-induced rat paw edema model is a frequently employed experimental model for evaluating acute inflammation in animals. Carrageenan started causing inflammatory reactions, such as oedema, when it was administered into the sub-plantar region of a rat's paw. Oedema is often noticeable within 30 minutes. A biphasic mechanism of action for the carrageenan-induced rat paw oedema model has indeed been identified. Bradykinin, serotonin, and histamine are released during the first phase, which lasts 1-2 hours, whereas prostaglandins are released during the second phase21, 22. The hydro-methanolic extract exhibited the most significant inhibition in paw volume at 3 hr, which suggests that the extract possibly possesses an inhibitory effect on prostaglandins release at the second phase.

Conclusion

Conclusively the current study results revealed that hydro-methanolic extract of the whole plant of *D.macraei* exhibited significant anti-inflammatory activity. These results validated the approved claims for the traditional uses of *D. macraei* in treating pain and inflammatory diseases. Further studies are required to separate the chemical constituents from the active hydro-methanolic extract, which exhibits marked anti-inflammatory activity. Additionally, research on the responsible mechanism for this activity is also required, which will authenticate its worth clinically.

Conflict of interest

All authors declare no conflict of interest.

Acknowledgment

The authors are thankful to IDMA Laboratories Ltd for providing all the required facilities to complete the study. The authors also thank the Department of Pharmacy, Banasthali University for their continuous support

References

1. Hassan FI, Abdulkadir UZ, Yaro AH, Danmalam UH. Analgesic, anti-inflammatory and antipyretic activities of the methanol leaf extract of *Dalbergia saxatilis Hook.F* in rats and mice. *Journal of Ethnopharmacol*ogy 2015;166:74-78.

2. Agli MD, Lorenzo C, Badea M. Plant food supplements with anti-inflammatory properties: a systematic review (1). *Critical Reviews in Food Science and Nutrition*. 2013;53(4):403-413.
3. Yanfen C, Shuhong T, Fanlin Z, Luwei X, Zhibin S. Antinociceptive and anti-inflammatory activities of *Schefflera octophylla* extracts. *Journal of Ethnopharmacology*. 2015;171:42-50.
4. Theuma P, Fonseca VA. Inflammation and emerging risk factors in diabetes mellitus and atherosclerosis. *Current Diabetes Reports*.2003;3:248–254.
5. Nickoloff BJ, Ben-Neriah Y, Pikarsky H. Inflammation and cancer: Is the link as simple as we think? *Journal of Investigative Dermatology*.2005;124:10-14.
6. Shaikh U, Pund M, Gacche RN. Evaluation of anti-inflammatory activity of selected medicinal plants used in Indian traditional medication system *in vitro* as well as *in vivo*. *Journal of traditional and complementary medicine*. 2016;4(6):355-361.
7. Viera IJ, Oliveira DB. Plants as a source of Anti-inflammatory agents. *Molecule*. 2020;25;1-22.
8. Ghasemian M, Owlia S, Owlia MB. Review of anti-inflammatory herbal medicines. *Advance Pharmacology Science*. 2016.
9. Kirtikar KR and Basu BD, Indian Medicinal Plants, Published by Lalit Mohan Basu, Allahabad, 1989;4 (2): 2401-2402.
10. Wood H, The Dendrobiums, A.R.G, Ganter Verlag Publishers, 2006.
11. Chhajed MR, Tomar GS, Gautam SP, Hariharan AG, Phytochemical Investigation and Evaluation of in-vitro Free Radical Scavenging Activity of *Flickingeria nodosa*Lindl., *Indian Journal of Pharmaceutical Education and Research*. 2008; 42(4).
12. Hui L, Shunmei Z, JiafuL ,Longfei Z , Yifeng Z , Xiujuan F, Siwei C , Hong N , Feifei L , Chunlian W, Dan Z, Antioxidant and anti-inflammatory activity of constituents isolated from *Dendrobium nobile* (Lindl.). *Frontiers in Chemistry*, 2022;1-12.
13. Kaushik P & Kishore N, Antibacterial potential of *Pholidota articulate* Lindl., A study in vitro. *Journal of the Orchid Society of India*.5, 93-96.
14. Zhang HJ, Zhou JJ & Li XS, Study advance of *Gastrodiaelata*b1. *Amino Acids Biotic Resources*. 2003; 25:17-20.
15. Nayan R,Bhalodia, Pankaj B,Nariya RN, Acharya VJ, Shukla. *In vitro* antioxidant activity of hydro alcoholic extract from the fruit pulp of *Cassia fistula* Linn., *Ayu journal*. 2013; 34 (2):209-214.
16. Ratheesh M, Helen A. Anti-inflammatory activity of *Ruta graveolens* Linn.on carrageenan induced paw oedema in wistar male rats. *African Journal of Biotechnology*. 2007; 6(10):1209-1211.
17. Khasin SM. and Rao PRM. Medicinal importance of orchids.*The Botanica*. 1999; 49:86-91.
18. Saroya, AS. Herbalism, phytochemistry and ethnopharmacology, Published by science publisher. 2010; 305.
19. Wealth of India, Raw Materials, D-E, CSIR, New Delhi, 1952; 3:43-44.