International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Evaluating the Anti-inflammatory Properties of *D. Hookeri* Rhizome Extract: Insights from an *In-Vivo* Study

Shubham Wani[1], Rekha Gour[2], Anant K. Patel[3]

[1]PG Student, Swami Vivekanand College of Pharmacy, Indore (M. P.), India

[2]Professor, Swami Vivekanand College of Pharmacy, Indore (M. P.), India
Corresponding Author Email: *gourrekha4[at]gmail.com*

[3]Professor, Swami Vivekanand College of Pharmacy, Indore (M. P.), India

Abstract: ***Inflammation occurs as defensive response, which include physiological adaptions to limit tissue damage and remove the pathogenic infections. The ethanolic rhizome extract of D. Hookeri was assessed for its in-vivo anti-inflammatory activity. The unfavourable consequences of synthetic medications for treating acute inflammatory diseases serve as a powerful incentive for scientists to create novel medications with fewer side effects. The soxhlation extraction technique was used to obtain ethanolic extract and the yield of the ethanolic extract of D. Hookeri's rhizomes was 28.27% w/w. The rhizomes of the ethanolic extract of D. Hookeri included alkaloids, saponins, tannins, flavonoids, glycosides, phenolic content, terpenoids, and volatile oils, according to the qualitative preliminary phytochemical screening. The anti-inflammatory efficacy of the ethanolic extract was evaluated against carrageenan induced rat paw edema at different doses (200 and 400mg/kg body weight) orally while indomethacin 10 mg/kg served as standard. The changes in the paw volume of rats were measured using mercury Plethysmometer. The significant anti-inflammatory activity was exhibited in the rats (p<0.05) by decreasing the paw edema volume. The % inhibition of paw edema by 400mg/kg body weight of the ethanolic extract of D. Hookeri extract at 5th hour was 38.14% while the Indomethacin treated rats showed 40.20% inhibition of paw edema. The study's findings support the use of this plant in the management of disorders associated with inflammatory diseases.***

Keywords: Inflammatory activity, Plethysmometer, rhizomes of *D. Hookeri*, Indomethacin

1. Introduction

The world is supplied with a rich abundance of therapeutic plants. Herbs have dependably been the primary type of drug in India. An immune system's defence against an infection or damage is inflammation. Inflammation serves to remove toxic and foreign stimuli and to rebuild tissue integrity and physiological function. There are two categories of inflammation: acute inflammation and chronic inflammation. If acute inflammation cannot be resolved, it might result in chronic inflammation, which can accelerate tissue damage and the ensuing functional consequences. It is one of the most prevalent and challenging diagnoses, and it typically affects regular individuals. The process of inflammation results from tissue injury, which also causes venule dilatation, an increase in vascular permeability, and the infiltration of cytokines, histamine, and other inflammatory substances. [1-4]

The mechanism of inflammation involves a series of events in which the metabolism of arachidonic acid plays an important role. It can be metabolized by Cyclooxygenase pathway to prostaglandins and thromboxane A2, or by the 5lipoxygenease pathway to hydroperoxyl-eicosatetraenoic acids (HPETE's) and leukotrienes, which are important biologically active mediators in a variety of inflammatory events. Upon appropriate stimulation of neutrophils, arachidonic acid is cleaved from membrane phospholipids and can be converted to leukotrienes and prostaglandins through 5-LOX or cox pathway respectively. Inhibition of 5-LOX and COX leads to decreased production of LT and PGs, such a drug would have potential to provide anti-inflammatory and analgesic effects with a reduction in GI side-effects. Reactive oxygen species are also a part of inflammatory processes, which are initiated by leukocyte activation. Therefore, screening of anti-oxidant properties may provide important information about the potential activity of a drug on inflammatory processes. [5, 6]

Inflammation occurs as defensive response, which include physiological adaptions to limit tissue damage and remove the pathogenic infections. Diseases caused by inflammation are an important factor of morbidity and mortality in humans. It is a fundamental pathologic process consisting of a dynamic complex of cytologic and chemical reactions that occur in the affected blood vessels and adjacent tissues (connective tissues) in response to an injury or abnormal stimulation caused by physical, chemical or biological agents resulting in;

1) The morphologic alterations brought upon by the local responses.
2) The destruction or removal of injurious material.
3) The responses that lead to repair and healing, the so called 'cardinal signs' of rubor (redness), calor (heat), tumour (swelling), dolar (pain). [5, 6]

D. Hookeri is a robust, rhizomatous perennial herb that may grow up to 45 cm long and belongs to the Asteraceae family. The Unani system of medicine (USM) has long employed the rhizomes of D. Hookeri in a range of dosage forms as a carminative, nervine tonic, and heart tonic (energizing and exhilarating). It has several heart-related properties, including being foetal-protective, antidotary, and very

helpful for palpitations. Usually, the rhizomes are used in compound compositions that provide body-tonic effects. The genus Doronicum has four subspecies and twenty-six species that are distributed over North Africa, Europe, and Asia. All perennial plants of this genus are rhizomatous. [7-15]

2. Material and Methods

Selection of plant

The herb's traditional medical uses led to its selection. [11, 12] Furthermore, phytochemicals found in D. *Hookeri's* ethanolic extract suggested that the plant has anti-inflammatory properties, which led us to choose the rhizomes of the plant for the intended purpose. [14]

Collection, identification and authentication of Plant material-

Plant rhizomes were collected from a nearby market in Indore, and they were verified by a botanist at APS University, Rewa, (M. P.).

Preparation of extracts

A Soxhlet extractor was used to carry out the extraction. The finely ground, air-dried rhizomes of *DoronicumHookeri* were subjected to a 24-hour soxhlation process, which involved a hot extraction utilizing ethanol as a solvent at a temperature of around 60°c. The extract was distilled in a porcelain evaporating dish and dried over a boiling water bath to produce a dark brown, semi-solid material. [14]

Phytochemical screening

Alkaloids, saponins, tannins, flavonoids, glycosides, phenolic content, terpenoids, and volatile oils were the main groups of phytochemicals that were qualitatively assessed in the ethanolic extract of *DoronicumHookeri* rhizomes.

Experimental Animals

Wistar rats, weighing 150-200g were obtained from the animal house of Swami Vivekanand college of pharmacy, Indore, India. The animals were kept individually in the large spacious hygenic cages at 22ºC±3ºC following 12 hours light and 12 hours dark cycle, allow them, free access to water *ad libitum* and food. Prior to being utilized in the research, the animals were given a week to acclimate. The experimental protocol was approved by the Institutional Animal Ethical Committee of our institute. (Approval No: IAEC/SVCP/2023/12) and were strictly in accordance with the norms of CPCSEA.

Acute oral toxicity [14]

The extensive literature review determined that the D. Hookeri rhizome extract was safe to use at doses of 300 mg/kg and 2 g/kg body weight. At both dosages, no mortality was seen. As a result, the plant's ethanolic extract was shown to have an LD50 of 2g/kg body weight.

Evaluation of Anti-inflammatory activity [16, 17]

Evaluation of anti-inflammatory activity of the extract was carried out using carrageenan induced paw edema in rats.

3. Experimental design

A total number of 20 healthy adult Wistar strain albino rats were divided into 5 groups each:

Table 1: Grouping of experimental animals

S. No.	Groups	No. of Animals
1.	Normal Control	2
2.	Negative Control	3
3.	Low dose of test extract (200 mg/kg)	5
4.	High dose of test extract (400 mg/kg)	5
5.	Standard Drug Indomethacin (10 mg/kg) [17]	5
	Total	20

Procedure [16-17]

Figure 1: Carrageenan Administration in Rats

The healthy Wistar rats were weighed and marked with ink just beyond the Tibio-tarsal junction on both the hind paw. Using the mercury displacement method, each rat's initial paw volume was recorded. All the rats except normal control were pre-treated orally with the test extract solution and standard drug. The first group served as normal control group and received normal saline orally. The second group served as negative control and inflammation was induced by injecting 1% carrageenan solution into the sub-plantar region of rat right hind paw. All the animals of 3rd test group were pre-treated with low dose of *D. Hookeri* (200mg/kg), after 1 hour the rats were injected with 1% carrageenan solution into the sub-plantar region of rat right hind paw. All the animals of 4th test group were pre-treated with high dose of *D. Hookeri (*400mg/kg), after 1 hour the rats were injected with 1% carrageenan solution into the sub-plantar region of rat right hind paw. The 5th group served as standard were pre-treated with Indomethacin (10mg/kg), after 1 hour the rats were injected with 1% carrageenan solution into the rat right hind paw.

The volume of paw edema was measured by using mercury Plethysmometer immediately after carrageenan injection at 0, 1, 2, 3, 4 and 5 hours. The average paw volume was measured and compared with control and standard groups. Reduction in the paw volume in *D. Hookeri* pre-treated groups compared with the control animals was considered as anti-inflammatory response.

Statistical analysis

The findings are given as mean ± S. E. M. One way analysis of variance (ANOVA) and the Turkey HSD test were used

for the statistical analysis. The results compared with those of the standard group and the normal saline treatment animal groups. Differences with values of P<0.05 were deemed significant for all tests.

4. Results

Extraction

The yield of the ethanolic extract of D. Hookeri's rhizomes was 28.27% w/w.

Qualitative phytochemical analysis of *Doronicum Hookeri* rhizomes extract

The ethanolic extract of *D. Hookeri's* rhizomes includes alkaloids, flavonoids, glycosides, volatile oils, saponins, tannins, and phenolic chemicals, according to the qualitative phytochemical study.

Anti-inflammatory response

Inhibition of paw edema in carrageenan induced paw edema in rats.

The *in-vivo* anti-inflammatory activity of *D. Hookeri* rhizomes ethanolic extract was compared with standard Indomethacin and control on an hourly basis up to five hours after the induction of inflammation. The results of anti-inflammatory activity were expressed in mean ± standard deviation of inflammation index and percentage edema inhibitory activity of rat paw edema, as shown in Table 1 and 2. It was found that a high dose of *D. Hookeri* rhizomes extract i. e.400 mg/kg has a greater inhibitory effect than a low dose i. e.200 mg/kg, with an increase in time. The rats were pre-treated with different dose level of ethanolic extract and Indomethacin, after 1 hour edema was induced by injecting carrageenan solution. During the search for anti-inflammatory efficacy of low dose and high dose level of *D. Hookeri* ethanolic extract, it was observed that Group-III (low dose 200mg/kg) % inhibition of paw edema at 5th hour was 32.47% whereas that of Group-IV (high dose 400mg/kg) at 5th hour was found to be 38.14% which when compared to Group-V (Indomethacin 10mg/kg at 5th hour was found to be 40.20%. The inhibition % of paw edema by 400mg/kg body weight of the extract was found statically significant ($p<0.05$) when it was compared with Indomethacin treated animals at 1, 2, 3, 4 and 5 hours. The highest reduction in the paw volume by the 400mg/kg body weight was 38.14% comparable to that of Indomethacin (40.20%) at 5th hour.

Percentage inhibition of paw edema by ethanolic test extract and standard drug.

The percentage of paw edema inhibition was calculated by using the following formula.

Inhibition of Paw edema (%) = Oc-Ot / Oc X 100.

Where 'Oc' is edema volume of control group and 'Ot' is edema volume of treated groups.

Table 2: Data % inhibition of rat paw edema by ethanolic extract from rhizomes of *D. Hookeri* and Indomethacin

		% of inhibition of paw edema					
Groups	Treatment	Dose	At 1st Hour	At 2nd Hour	At 3rdHour	At 4th Hour	At 5th Hour
Group-II	Carrageenan	0.1% carrageenan	0	0	0	0	0
Group-III	Ethanolic extract	200mg/kg	7.26	13.58	18.18	23.56	32.47
Group-IV	Ethanolic extract	400mg/kg	8.93	17.39	24.59	31.41	38.14
Group-V	Indomethacin	10mg/kg	10.61	18.47	26.73	33.50	40.20

Note: values are % inhibition of rat paw edema over standard group, $p<0.05$, (One-way ANOVA followed by Tukey HSD test) compared to the control group

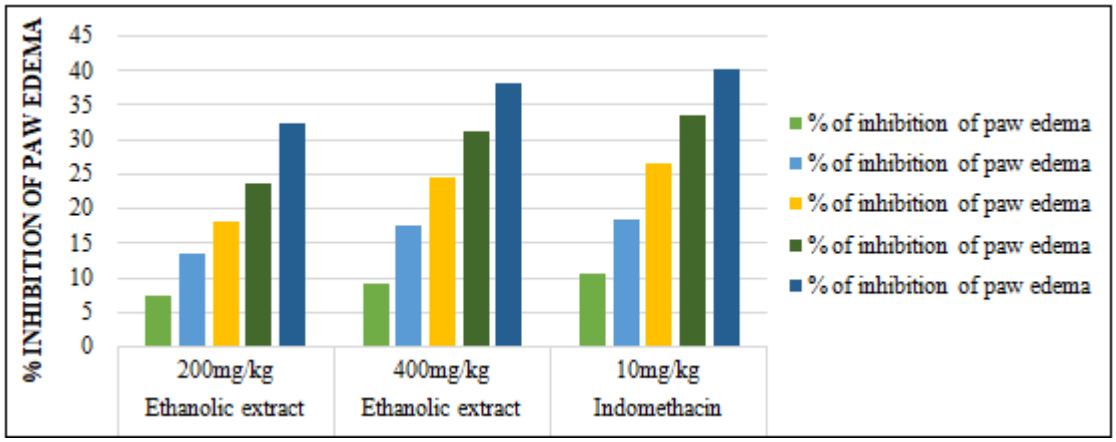

Figure 2: % inhibition of paw oedema of rats at different time intervals of ethanolic extract of *D. Hookeri* relative to the control group

5. Discussion

In plants, comprising medicinal plants, fruits, vegetables, flowers, leaves, roots, and fibers, phytochemicals are naturally occurring bioactive substances that function as a defensive mechanism against disease or, more precisely, as a means of disease protection for plants. The rhizomes of the *Doronicum Hookeri* have been used traditionally in Unani system of medicine to cure various ailments largely cardiovascular disorder. The anti-inflammatory activity of ethanolic extract of rhizomes was evaluated for *in-vivo* anti-inflammatory activity in rats. The present study reported

significant anti-inflammatory activity against carrageenan induced paw edema in rats. Soxhlet apparatus was used to obtain ethanolic extract employing ethanol as a solvent. In this method, a low dose and high dose (200 and 400 mg/kg) of ethanolic extract of rhizomes of *D. Hookeri* was used as test group and the significant anti-inflammatory activity was evaluated comparing the test group rats with standard group Indomethacin (10 mg/kg) treated rats. The ethanolic extract at 400mg/kg showed significant ($p<0.05$) % inhibition of rat paw edema. The ethanolic extract 400mg/kg treated rats inhibited 31.41% and 38.14% rat paw edema at $4^{th}$and 5^{th} hour while Indomethacin 10mg/kg treated rats showed 33.50% and 40.20% inhibition of rat paw edema in carrageenan induced rat paw edema.

6. Conclusion

The present study reported that the ethanolic extract of rhizomes of *D. Hookeri* exhibits anti-inflammatory activity. The anti-inflammatory activity may be attributed to the presence of phytochemicals such as alkaloids, saponins, tannins, flavonoids, glycosides and phenolic content, present in the ethanolic extract of the rhizomes of *D. Hookeri.* Carrageenan induced paw edema in rats is most commonly and widely used anti-inflammatory model for evaluating anti-inflammatory activity. The results of this investigation indicate that the ethanolic extract 400mg/kg has a marked 38.14% inhibition of rat paw edema at 4^{th} and 5^{th} hour as compared to standard group.

Acknowledgement
Authors are very grateful to Mentor Mrs. Rekha Gour and Swami Vivekananda College of Pharmacy, Indore, India for providing the facilities for the research.

References

[1] Syed Ziaur Rahman, Rahat Ali Khan and Abdul Latif, "Importance of pharmacovigilance in Unani system of medicine", Indian Journal of pharmacology, 2008; 40 (1): S-17-S-20.
[2] Dwivedi, T., Kanta, C., Singh, L. R., & Prakash, I. (2019). A list of some important medicinal plants with their medicinal uses from Himalayan State Uttarakhand, India. Journal of Medicinal Plants, 7 (2), 106-116.
[3] Vijayalakshmi R., Ranganathan R. "Ethnobotanical Studies on Some Traditional Medicinal Plants in Cuddalore District", IJPC Drug Invention Today, 2011, 3 (7): 160-164.
[4] C. K. Kokate, A. P. Purohit, S. B. Gokhale. Pharmacognosy. Nirali Prakashan, 53^{rd} edition, 2017. Pg. no.7.1-7.46.
[5] Hardman JG, Limbird LE, Gilman AG, editors. Goodman and Gilman's, The pharmacological basis of therapeutics. McGraw-Hill Medical publishing division. Edition 10^{th}, 2001. Pg no.687-731.
[6] H. P. Rang, M. M. Dale, J. M. Ritter, R. J. Flower. Rang and Dale's Pharmacology. Churchill Livingstone edition 6^{th}, 2007. Pg. no.226-247.
[7] Mohammad Avid, Riyaz Ahmad, Mohd Afsahul Kalam, Faheem Akhtar, Wasim Ahmad, "*DarunajAqrabi* (*DoronicumHookeri* hook f.): potential medicinal rhizome of Unani medicine-a review, International
[8] Journal of Creative Research Thoughts, 2023, 11 (6): 647-657.
[9] Asif Iqbal, M. Akhlaq, Shiraz M. Siddiqui, Muhammad K. Alum, *et. al.*, "Ethnopharmacological and medicinal review on *DoronicumHookeri* Clark C. B & J. D Hooker", Hamdard Medicus, 2022, 65 (1): 51-62
[10] Aiman Faheem, Aisha Siddiqui, Shahid Shah Chaudhary, Mohd Aslam, M Mujeeb, Nuzhat Praween, "Ethnobotany and pharmacognosy of *DarunajAqrabi* (*DoronicumHookeri* Hook. F.) with special reference of Unani medicine", International Journal of Botany Studies, 2021; 6 (1): 449-452.
[11] Natale Badalamenti, Aurora Modica, Vincenzo Ilardi, and Maurizio Bruno., "Chemical Constituents and Biological Properties of Genus Doronicum", chemistry and biodiversity, 2021; 18 (12): 1-18.
[12] Mohd Aleem, Mohd Imran Khan, Qamrul Islam Usmani, Altaf Ahmad, "Review on *Darunaj-Aqrabi* (*DoronicumHookeri* C. B. Clarke): an Unexplored Medicinal Plant of Unani System of Medicine". Journal of Ayush: Ayurveda, Yoga, Unani, Siddha and Homeopathy, 2020; 9 (2): 41–52.
[13] Mohd. Afsahul kalam, Ghufran Ahmad, Anwar Shahzad, Shaikh Ajij Ahmad Maqbul, and Mohd Sayeed Akhtar, "Therapeutic potential of rhizomatous plant used in Unani Medicare system", Natural bioactive compounds, 2019; 409-464.
[14] Shameem A. Rather, Abdul N. Ansari, Mansoor A. Siddiqui, "*DaroonajAqrabi*-A review, TANG [Humanitas Medicine], 2017; 7 (4): 1-2.
[15] S. N. Syed, W. Rizvi, A. Kumar, A. A. Khan, S. Moin and P. A. Khan, "Study to Evaluate the Antioxidant and Hepatoprotective Activities of Roots Extracts of *DoronicumHookeri* in CCl4 Treated Rats", European Journal of Medicinal Plants, 2014; 4 (6): 675-685.
[16] Deepika Gupta, Bruce Bleakley and Rajinder K. Gupta, "Phytochemical analysis and antioxidant activity of herbal plant *DoronicumHookeri* Hook f. (Asteraceae) ", Journal of Medicinal Plants Research, 2011; 5 (13): 2736-2742.
[17] Ismail S. M., K. R. S. Sambasiva Rao, Matcha Bhaskar, "Evaluation of anti-inflammatory activity of *BoswelliaSerrata* on carrageenan induced paw edema in albino Wistar rats", International Journal of Research in Medical Sciences, 2016; 4 (7): 2980-2986.
[18] Yogendra Singh Chouhan, H. C. Kataria and C. S. Goswami, "Anti-inflammatory activity of methanolic extract of *BrassicaJuncea* seed on carrageenan induced paw edema in rats", International journal of sciences and research, 2014; 5 (9): 3849-3851.
[19] Abhishek Kumar Pandey, Preeti Pande, "Evaluation of phytochemicals, anti-inflammatory and antioxidant potential of *AegleMarmelos* L. leaves", Advances in Pharmacology and Pharmacy, 2022; 11 (1): 66-67.
[20] Aisha O. Musa, Habatullah K. Usman, Irisim Titus, Amina B. Olorukooba, Abubakar B. Aliyu, Hadiza Bello, "Analgesic and anti-inflammatory studies of methanol stem bark extract of *BurkeaAfricana* Hook (Fabaceae) ", Tropical Journal of Natural Product Research, 2018; 2 (8): 375-379.

[21] Mohd. Afsahul kalam, Ghufran Ahmad, Anwar Shahzad, Shaikh Ajij Ahmad Maqbul, and Mohd Sayeed Akhtar, “Therapeutic potential of rhizomatous plant used in Unani Medicare system”, Natural bioactive compounds, 2019; 409-464.