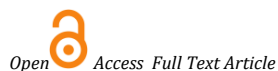


Available online on 15.01.2024 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

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Research Article

In-vivo Pharmacological Evaluation of Analgesic Activity of Ethanolic Extract of *Doronicum hookeri* Clarke Rhizomes in Wistar Rats

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Article Info:



Article History:

Received 19 Oct 2023
Reviewed 05 Dec 2023
Accepted 23 Dec 2023
Published 15 Jan 2024

Cite this article as:

Wani S, R Gour, Patel AK, *In-vivo* Pharmacological Evaluation of Analgesic Activity of Ethanolic Extract of *Doronicum hookeri* Clarke Rhizomes in Wistar Rats, Journal of Drug Delivery and Therapeutics. 2024; 14(1):9-12

DOI: <http://dx.doi.org/10.22270/jddt.v14i1.6168>

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Abstract

A number of plants are used traditionally in different medical conditions by practitioners of traditional medicines but their claim is not yet evaluated scientifically in laboratory animals. Based on the ethnomedical claims made by the Unani health practitioners, the Clarke rhizomes of *Doronicum Hookeri* was evaluated for analgesics activity in rats to confirm its claim. The successive plant extraction was carried out using Soxhlet apparatus by employing ethanol as a solvent of the dried rhizomes of *Doronicum Hookeri*. The % yield of extracted compound was 28.27% w/w. The qualitative preliminary phytochemical screening revealed the presence of alkaloids, saponins, tannins, flavonoids, glycosides, phenolic content, terpenoids and volatile oils in the rhizomes of ethanolic extract of *D. Hookeri*. The extract was orally administered in rats at low dose and high dose (200 mg/kg, 400 mg/kg) and was evaluated using Eddy's Hot Plate Method in rats for analgesics activity. Diclofenac sodium (50 mg/kg) and was taken as a standard drug. The maximum possible analgesia % of ethanolic extract of rhizomes of *D. Hookeri* were found to be highest at a dose of 400mg/kg which showed 68.95% and 80% analgesia at 45 minute and 60 minute which when compared to Diclofenac sodium showed 73.28% and 85.71% at 45 minute and 60 minutes. The analgesic efficacy of ethanolic extract of rhizomes of *D. Hookeri* showed significant analgesic activity ($p < 0.04$) in dose dependent manner at different time intervals of 0, 15, 30, 45 and 60 min. This asserts ethnomedical claims of some tribes regarding the analgesic activity of this plant.

Keywords - Analgesics, eddy's hot plate, *D. Hookeri*, Diclofenac sodium.

INTRODUCTION

Nature always stands as a golden mark exemplifies the outstanding phenomena of symbiosis. The biotic and abiotic elements of nature are all inter-dependent. Plants are indispensable to man. Because of man's natural curiosity, knowledge about medications has collected over thousands of years, and as a consequence, we now have numerous efficient ways to provide healthcare. Herbal medicinal products are a kind of medicine that uses roots, stems, leaves, flowers, or seeds of plants to enhance health, prevent sickness, and treat illness. Herbal medicine, often known as herbalism, is the study of pharmacognosy and the application of therapeutic plant usage, which forms the foundation of conventional medicine.¹⁻³

Pain is a disagreeable feeling and emotional reaction to real or anticipated tissue injury. Pain arises when anything hurts and causes a discomfort or unpleasant sensation. Increased vascular permeability, increased protein denaturation, and altered membranes are only a few of the complicated events that make up inflammation, which is often accompanied by discomfort. Non-steroidal mitigating drugs generally shortened to NSAIDs, are a medication class that bunches

together medications that decline agony and lower fever and in higher dosage decline irritation. Natural remedies have the potential to treat various diseases including pain and inflammation. Herbal drugs or their extract plays a vital role in enhancing the human health.⁴

D. Hookeri belonging to family Asteraceae is a rhizomatous herbaceous perennial stout herb, up to 45cm long. The rhizomes of *D. Hookeri* have traditionally been used in Unani system of medicine (USM) in a variety of dose form as a cardiac tonic (invigorating and exhilarating), nervine tonic, and carminative. Fetus-protective, antidotary etc. it is highly beneficial for palpitation and is a component of certain heart related medications. The rhizomes are typically utilized in compound formulations with tonic benefits on the body. There are 26 species and 4 subspecies in the genus *Doronicum*, which can be found throughout Asia, Europe, and North Africa. This genus encompasses entirely rhizomatous, perennial herbs. The medicinal values of these plants lie in bioactive phytochemical constituents with the intention of produce exact physiological actions on the human and animal body.⁵⁻¹³

MATERIAL AND METHODS

Selection of plant

The herb was chosen based on its historical medicinal claims.⁹ Also, the presence of phytochemicals in the ethanolic extract of *D. Hookeri* was suggestive of the analgesics activity which encouraged us to select *D. Hookeri* rhizomes for the proposed activity.¹²

Collection, identification and authentication of Plant material

Rhizomes from plants were gathered from an Indore local market, and were authenticated by a botanist at APS University, Rewa (M.P.), India.

Preparation of extracts

The extraction was carried out utilizing Soxhlet extractor. The air-dried fine powder of the rhizomes of *Doronicum Hookeri* were exposed to soxhlation for 24 hours by hot extraction at around 60°C at room temperature using ethanol as a solvent. The extract was distilled in the porcelain evaporating dish and was evaporated to dryness on boiling water bath to achieve dark semi-solid brown mass.¹²

Phytochemical screening

Qualitative assessment of ethanolic extract of rhizomes of *Doronicum Hookeri* was performed for major classes of phytochemicals namely alkaloids, saponins, tannins, flavonoids, glycosides, phenolic content, terpenoids and volatile oils.

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 hygienic cages at 22°C±3°C following 12 hours light and 12 hours dark cycle, allow them, free access to water *ad libitum* and food. The animals were allowed to acclimatize for seven days before being used for the studies. 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

The *D. Hookeri* rhizome extract was shown to be safe to use at dosages of 300 mg/kg and 2 g/kg body weight after a thorough study of the literature. There was no mortality at either dose. The ethanolic extract of the plant was therefore found to have an LD50 of 2g/kg body weight.

Grouping of experimental animals

Healthy Wistar albino rats of either sex were used for the study. After one week of acclimatization, the rats were divided into four groups comprising total 17 rats in different groups of animals.

Group A- Normal control

Dose: Normal saline (0.9% NaCl) 1 ml/100g

Group B-Low dose of ethanolic extract

Dose: 200 mg/kg

Group C-High dose of ethanolic extract

Dose: 400 mg/kg

Group D- Standard group

Dose: Diclofenac Na (50mg/kg)^{14,15}

Evaluation of Analgesics Activity

Eddy's hot plate method

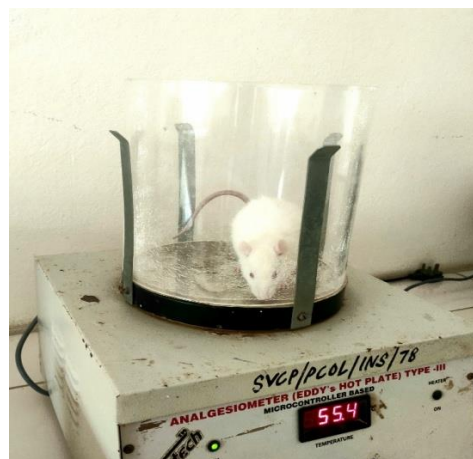


Figure 1: Determination of Analgesic Activity in Rats.

The rats were weighed and marked individually. All the rats except normal control was pre-treated with low dose and high dose of ethanolic extract of *D. Hookeri* (200mg/kg; 400mg/kg) and standard preparation of diclofenac sodium (50mg/kg). The test solution and the reference drug were constituted in normal saline as vehicle and were administered orally through oral gavages. The volume of the drug and the test solution to be administered was calculated based on the body weight of animals following OECD guidelines. Group1 received normal saline and was normally kept. Group 2 received normal saline and were placed on hot plate maintained at 55°C within the restrainers. The reaction time (in seconds) or latency period was determined as licking their paw or jumping. The treatment group 3 and 4 was pre-treated with low dose (200mg/kg) and high dose (400mg/kg) of ethanolic extract of *D. Hookeri* and the reaction time was recorded at initial (0 min) and at 15, 30, 45 and 60min. interval. The cycle was repeated with all the animals of the particular group and the reaction time was recorded. The 5th treatment group was administered the reference standard drug Diclofenac sodium (50mg/kg) orally through the oral gavages and the reaction time was recorded. The cycle was repeated with all the animals of the treatment group. The maximum reaction time was fixed at 45 sec to prevent any injury to the tissues of the paws. If the reading exceeds 45 sec, it would be considered as maximum analgesia. The results of the test extract of low dose, high dose and negative control were compared with the normal control group and standard reference drug Diclofenac sodium.¹⁴⁻²⁰

The maximum possible analgesia (MPA) will be calculated as follows¹⁶

$$MPA = \frac{\text{Reaction time for treatment} - \text{reaction time for saline}}{45 \text{ sec} - \text{reaction time for saline}} \times 100$$

Statistical Analysis

The results are reported as mean ± S.E.M. The statistical analysis were performed using one way analysis of variance (ANOVA) followed by Turkey HSD test. The outcomes were compared to those of the normal saline treated and standard group. For all tests, differences with values of P<0.04 were considered significant.

RESULTS

Extraction

The % yield of ethanolic extract of rhizomes of *D. Hookeri* extracted compound was 28.27% w/w.

Qualitative phytochemical analysis of ethanolic extract of *Doronicum Hookeri* rhizomes

The qualitative phytochemical analysis indicated that the ethanolic extract of rhizomes of *D. Hookeri* contains alkaloids, flavonoids, glycosides, volatile oils, saponins, tannins and phenolic compounds.

Analgesic activity

Hot plate reaction time in rats:

While evaluating the analgesics activity of the ethanolic extract using hot plate, it was observed that normal 0.9% NaCl solution (group-1) did not have any significant changes in basal reaction time, the low dose and high dose of ethanolic extract of rhizomes of *D. Hookeri* showed highly significant effect at 15, 30, 45 and 60 min as compared with standard group. The ethanolic extract at a dose of 400mg/kg was found to have significant difference in basal reaction time at different time period. The ethanolic extract at a dose of 400mg/kg showed peak analgesic effect 39.8 ± 0.18 at 60 min when compared to diclofenac sodium at 60 min.

Table 1: % Maximum Possible Analgesia in *D. Hookeri* and Diclofenac sodium treated rats.

Maximum Possible Analgesia (%)							
Group name	Treatment	Dose	0 min	15 min	30 min	45 min	60 min
Test 1	Ethanolic extract	200mg/kg	17.60	33.57	42.96	51.62	60.00
Test 2	Ethanolic extract	400mg/kg	22.09	47.14	57.40	68.95*	80.00*
Standard	Diclofenac Na	50mg/kg	23.59	50.71	61.01	73.28	85.71

Note: Values are % Maximum Possible Analgesia over standard group(n=5). * $p < 0.04$, (One-way ANOVA followed by Turkey HSD test) compared to the control group

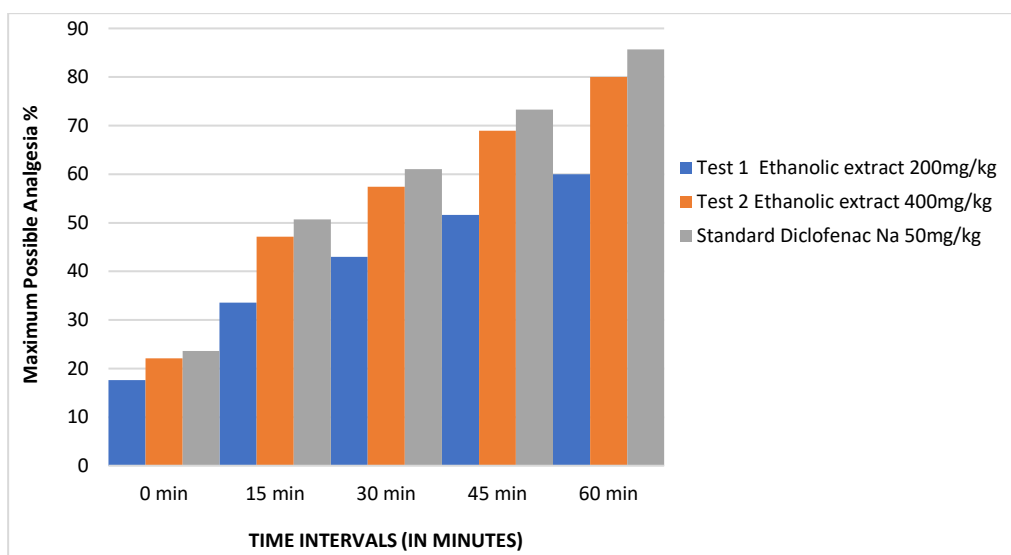


Figure 2: Increase in reaction time of rats jumping reaction at different time intervals of ethanolic extract of *D. Hookeri* relative to the control group.

DISCUSSION

Unani medicine (also called as Greco-Arab medicine) is an ancient system of medicine originated from Greece. *Darunaj Aqrabi* (*Doronicum Hookeri* Hook. f) is one of the roots to be used as medicinal importance in Unani medicine. The analgesics efficacy of ethanolic extract of rhizomes of *Doronicum Hookeri* was assessed *in-vivo* in eddy's hot plate technique in rats. The present study demonstrated a significant analgesic activity at different dose level. The extraction of the powdered rhizomes of *D. Hookeri* was carried out using ethanol as a solvent in the Soxhlet apparatus. The analgesics activity of ethanolic extract of *D. Hookeri* was studied by Eddy's Hot Plate method which is used to determine central analgesics activity. In the Hot Plate Method, the two doses of ethanolic extract of *D. Hookeri* showed significant analgesic activity when compared to control and standard Diclofenac sodium treated group. In our study, EDH

(200 and 400 mg/kg, p.o) significantly elevated ($p < 0.04$) mean basal reaction time in hot plate method. The ethanolic extract of the rhizomes exhibited analgesic activity in Hot Plate method of nociception and exerted its effect through central pain pathway.

CONCLUSION

The present experimental protocol showed that the rhizomes of *D. Hookeri* elicited a significant analgesic activity in centrally acting analgesics model of Eddy's Hot Plate method. The ethanolic *D. Hookeri* rhizomes extract showed analgesic property to those observed for NSAIDs, such as Diclofenac sodium. The ethanolic extract showed significant analgesic effect ($p < 0.04$) when compared to control group. However, further studies are needed to isolate and characterize analgesics chemical constituents present in ethanolic extract of the rhizome.

Acknowledgement

Words cannot express my gratitude to my professor Mrs. Rekha Gour for her invaluable patience and feedback. I also could not have undertaken this journey without my guide, who generously provided knowledge and expertise. This endeavour would not have been possible without the blessings of my parents which lead me towards achieving my goals.

Funding source: Educational study

Competing interests/Conflicts of interest: The authors have No Conflicts of interest regarding this investigation.

Ethical approval for animal: 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.

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