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Original Article

Pharmacology Section

Evaluation of Analgesic Activity of Extracts of *Delphinium denudatum* in Animal Models: A Dose-dependent Pre-clinical Trial

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ABSTRACT

Introduction: Pain is a common symptom of majority of clinical disorders which brings patients to a physician. Many traditional medicines have been introduced for relieving the pain and one such herb, *Delphinium denudatum*, is claimed to have an analgesic effect.

Aim: To evaluate the analgesic activity of ethanolic extract and methanol fraction of *Delphinium denudatum* on Wistar albino rats.

Materials and Methods: This experimental study was carried out in the Department of Pharmacology, JN Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. The analgesic activity was evaluated by employing the Eddy's Hot Plate method and Tail Flick responsemethod (Orchid Scientifics, India). In both the tests, Rats of either sex weighing 150-200 g were used. The total number of animals n=36 were allocated to six groups consisting of six animals each. Group I received propylene glycol 0.3 mL/100 g p.o., Group II received pentazocine 30 mg/kg i.p, Group III received ethanolic extract of *Delphinium denudatum* 300 mg/kg p.o., Group IV received ethanolic extract of *Delphinium denudatum* 600 mg/kg p.o., Group V received methanol fraction of *Delphinium denudatum* 200 mg/kg p.o., Group VI received methanol fraction of *Delphinium denudatum* 400 mg/kg p.o. The response noted in animals who were tested by hot plate method, was reaction time for licking/biting of both the paws before and after administration of control and test drugs. However, in Tail flick test, the pain threshold response was recorded before and after administration of control and test drugs. The statistical analysis was done by using one-way ANOVA. The data are expressed as Mean±SEM. $p<0.05$ was considered to be statistically significant.

Results: A significant analgesia was produced in all the treatment groups when compared with control group in both the test models. A dose-dependent significant ($p<0.001$) analgesia was recorded in all the groups received ethanolic extracts and *methanol* fraction of *Delphinium denudatum*. However, a significant increase in reaction time and pain threshold in both the test models was observed in the groups who were given a higher dose of ethanolic extracts and *methanol* fraction of *Delphinium denudatum*. Interestingly, this study noted an approximately parallel degree of significant analgesia with a group who received extract of *Delphinium denudatum* in a dose of 600 mg/kg to a pentazocine group.

Conclusion: The present study reveals the dose-dependent significant analgesic activity of the extracts of *Delphinium denudatum* in both the test. However, the degree of analgesia was recorded significantly higher in groups that received higher doses of extracts of *Delphinium denudatum*.

Keywords: Analgesia, Experimental study, Medicinal herb

INTRODUCTION

Pain is one common health problem with substantial socio-economic impact because of its high incidence. It is associated with a number of diseases and is estimated that 80-100% of the population experience back pain at least once in the life-time [1]. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are the mainstay of treatment of pain [2]. It was reported that the risk of gastrointestinal bleeding was significantly associated with the acute use of NSAIDs like regular-dose aspirin, diclofenac, ketorolac and Piroxicam etc., increased the risk of bleeding in both acute and chronic therapy [3]. Therefore, it is necessary to search for new drugs with less adverse effects. Medicinal plants have been used in the development of new drugs and continue to play an invaluable role in the progress of drug discovery [4]. Recently, many natural medicines derived from medicinal plants such as *Capsicum annuum*, *Cannabis sativa* and *Papaver somniferum* were considered as effective and safer for treatment of various diseases including pain [5].

Delphinium denudatum family Ranunculaceae is a medicinal herb commonly known as *jadwar* and used in Unani Medicine. The roots are reported to be useful in a variety of ailments such as paralysis, epilepsy, facial palsy, insanity, mania, hysteria, atony, migraine, numbness, tremors, infantile convulsions, aconite poisoning, snake bite, scorpion sting, arthritis, cardiac weakness, palpitation, rheumatism, toothache. Delphinium plants have been medicinally used for centuries [6,7]. Use of its root as an analgesic is found in Unani medicine [8]. Earlier studies showed that ethanolic extract of *Delphinium denudatum* has analgesic activity [9,10].

The present study was performed to validate the earlier study and to screen additionally the effect of its methanol fraction.

MATERIALS AND METHODS

This experimental study was carried out in the Department of Pharmacology, JN Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India, from July 2013 to January 2016. The study followed ARRIVE guidelines and was approved by the Animal Ethics Committee. (Registration No. 401/RO/C/2001/CPCSEA).

Plant Materials

Roots of *Delphinium denudatum* were obtained from the local market of Aligarh. These were identified and authenticated by Chief Scientist, Raw Material Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. A sample specimen of plant material was deposited in the NISCAIR.

Preparation of Extract

The Delphinium denudatum roots procured and shade-dried. Plant material (100 g) was powdered by using an electrical grinder (REMI

auto-mix blender, Vasai, India). The powdered plant material was extracted with 300 mL of absolute alcohol by employing Soxhlet apparatus. The plant extract was filtered, evaporated and dry at 40°C on a water bath. Thus the extracted material (semisolid mass) was weighed to calculate its yield in percentage. The final yield of ethanolic extract of *Delphinium denudatum* was 4.4%.

Preparation of Fraction

Dried roots of *Delphinium denudatum* were crushed and one kilogram powder was made. The powder was air-dried and exhausted with 95% ethanol and the solvent was isolated by steam distillation. Under reduced pressure, the extract was concentrated to dark gummy mass. The residue so obtained was fractionated by refluxing with benzene, ethyl acetate and methanol, consecutively. The final yield of the methanol fraction of *Delphinium denudatum* was 3.2%.

Animals Used

Wistar albino rats, procured from the Central Animal House, weighing between 150-200 g of either sex were used. They were housed in polypropylene cages bedded with husk and provided with standard pellet diet (Ashirwad Industries, Chandigarh) and water ad libitum. The animal room was maintained at a temperature of 18- 29°C, humidity 30-70%, 12 hours light/dark cycle. Rats were acclimatised to the laboratory condition for one week prior to experimental use.

Acute Toxicity Study

An ethanolic extract of plant material is claimed to be safe. However, the limit toxicity of methanol fraction of *Delphinium denudatum* in addition to acute toxicity in accordance to Organisation for Economic Cooperation and Development (OECD) Guidelines 425" was done on healthy adult female rats (100-150 g). A dose of 2000 mg/ kg was administered to a group of five animals for calculation of LD50 of methanol fraction and they were observed for 14 days. No mortality related above limit toxicity dose was noted [11].

Drugs

Pentazocine lactate (Inj. Fortwin, Ranbaxy Lab. Ltd., India).

EXPERIMENTAL PROTOCOL

Analgesic Activity by Eddy's Hot Plate Method

The present study was performed by using Eddy's hot plate method (Orchid Scientifics, India). The hot plate is an electrically heated aluminium plate with a temperature ranging between 55°C to 56°C.

Rats of either sex (150-200 g) were used. The response noted was the reaction time of licking/biting of both paws. They were similarly screened and those responding in <6 seconds were chosen. The paws of rats are very sensitive to heat at temperatures compared to skin.

The selected animals were placed on hot plate to record the response. The reaction time was measured at the interval of 30, 60, 90, 120, 150, 180, 210 and 240 minutes after the administration of control and test drugs. Propylene glycol 0.3 mL/100 g p.o. served as control whereas pentazocine 30 mg/kg i.p. was administered as standard drug. The cut-off time for response reaction was 30 seconds. The plate was wiped clean every time with saline if urination/defecation was found. The test was done in six groups as shown in [Table/Fig-1].

Groups	Medication
Group I (Normal control)	Propylene glycol 0.3 mL/100 g p.o.
Group II (Standard control)	Pentazocine 30 mg/kg i.p
Group III (EEDD300)	Ethanolic extract of *Delphinium denudatum* 300 mg/kg p.o.
Group IV (EEDD600)	Ethanolic extract of *Delphinium denudatum* 600 mg/kg p.o
Group V ((MFDD200)	Methanol fraction of *Delphinium denudatum* 200 mg/kg p.o.
Group VI (MFDD400)	Methanol fraction of *Delphinium denudatum* 400 mg/kg p.o.

[Table/Fig-1]: Experimental Design

Analgesic Activity by Rat Tail Flick Test

The method is based upon the reaction of rats to heat stimulus applied to their tail. It was performed by using the analgesiometer (Orchid Scientifics, India). Rats of either sex (150-200 g) were placed in restraining holder so that the tail between the hole and tail tip or single point 3-5 cm from the tip of tail are directly kept over heated nichrome wire. The time taken by the rats to withdraw the tail was recorded. Heat intensity was adjusted such that the average withdrawn latency is 3-6 seconds and a maximum cut-off time of 15 seconds adopted to prevent undue tissue damage. Tail flick latency was tested at 30 minutes interval for four hours. The test was done in the groups as shown in [Table/Fig-1].

STATISTICAL ANALYSIS

The statistical analysis was done by using one-way ANOVA. The data is expressed as Mean±SEM. $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of Ethanolic Extract of *Delphinium denudatum* Root on Reaction Time in Rats on Eddy's Hot Plate

The rats in the control group responded within the cut-off time of six seconds in all time periods [Table/Fig-2]. The Group II (Standard control) showed significant ($p<0.001$) increase in reaction time (seconds) as 5.48, 7.05, 8.13, 8.29, 6.96 and 5.85 at the interval (minutes) of 30, 60, 90, 120, 150 and 180, respectively. Ethanolic extracts in low (Group III/EEDD300) and high (Group IV/600 mg/kg) doses showed significant increase in reaction time (seconds) as 4.50 ($p<0.05$), 4.90 ($p<0.001$), 5.71 ($p<0.001$), 5.47 ($p<0.001$), 4.84 ($p<0.01$) and 4.97 ($p<0.001$), 5.41 ($p<0.001$), 6.36 ($p<0.001$), 5.88 ($p<0.001$), 5.35 ($p<0.001$), respectively, at the interval (minutes) of 60, 90, 120,150 and 180. It was further noticed that mean reaction time was higher in the (Group IV/600 mg/kg). The peak effect in both groups was seen at 120 minutes.

Effect of Ethanolic Extract of *Delphinium denudatum* Root on Reaction Time in Rats on Tail Flick Test

The rats in the control group (Group I/Normal control) responded within the cut-off time of six seconds in all time periods [Table/Fig-3]. The Standard pentazocine group (Group II/Standard control)

Group	0 minute	30 minutes	60 minutes	90 minutes	120 minutes	150 minutes	180 minutes	210 minutes	240 minutes
Normal Control	3.89±0.1	3.76±0.1	3.89±0.1	3.89±0.1	3.95±0.2	3.90±0.1	3.89±0.1	3.88±0.1	3.85±0.1
Standard Control	3.53±0.2	5.48±0.4***	7.05±0.2***	8.13±0.2***	8.29±0.3***	6.96±.39***	5.85±0.4***	4.24±0.4	3.54±0.1
EEDD 300	3.83±0.09	4.01±0.1	4.50±0.2*	4.90±0.1***	5.71±0.1***	5.47±0.09***	4.84±0.06**	4.07±.09	3.82±0.10
EEDD 600	3.93±0.05	4.20±0.03	4.97±0.03***	5.41±0.07***	6.36±0.0***	5.88±0.05***	5.35±0.06***	4.25±0.07	3.91±0.04

[Table/Fig-2]: Effect of ethanolic extract of *Delphinium denudatum* root on reaction time in rats on Eddy's hot plate.
Reaction Time: Mean±SEM (n=6) sec. *p<0.05, **p<0.01,***p<0.001 as compared to normal control. EEDD: Ethanolic extract of Delphinium denudatum. (300 and 600 mg/kg)

showed significant (p<0.001) increase in reaction time (seconds) as 5.92, 6.67, 8.15, 9.02, 7.79, 6.73 and 5.01 at the interval (minutes) of 30, 60, 90, 120,150, 180 and 210 respectively. Ethanolic extract in low dose (Group III/EEDD300) showed significant increase in reaction time (seconds) as 5.12 (p<0.001), 5.43 (p<0.001), 5.82 (p<0.001), 5.50 (p<0.001), 5.11 (p<0.01) at the interval (minutes) of 60, 90, 120,150 and 180 respectively. Ethanolic extract in high dose (Group IV/EEDD600) showed statistically significant (p<0.001) increase in reaction time (seconds) as 5.31, 5.75, 6.52, 5.83, 5.03, 4.46 at the interval (minutes) of 60, 90, 120,150, 180 and 210 respectively. It was further noticed that pain threshold was higher in the high dose group (Group IV/EEDD600). The peak effect was seen at 120 minutes in both groups.

Effect of Methanol Fraction of *Delphinium denudatum* Root in Rats on Eddy's Hot Plate Test

The rats in the control group (Group I/Normal control) responded within cut-off time of six seconds in all time periods. The Group II/ Standard control showed statistically significant (p<0.001) increase in reaction time (seconds) as 5.95, 7.22, 8.39, 8.77, 7.46 and 6.41 at the interval (minutes) of 30, 60, 90, 120, 150 and 180, respectively. Group V/MFDD200 showed statistically significant increase in reaction time (seconds) as 4.98 (p<0.01), 5.87 (p<0.001), 5.59 (p<0.001), 5.00 (p<0.001), at interval of 90, 120, 150 and 180 (minutes). However, Group VI/MFDD400 showed statistically significant increase in reaction time (seconds) as 5.32 (p<0.01), 5.99 (p<0.001), 6.54 (p<0.001), 6.04 (p<0.001), 5.07 (p<0.01) respectively, at interval of 60, 90, 120,150 and 180 [Table/Fig-4]. It was further noticed that mean response time was higher in the large dose group (Group VI/MFDD400). The peak effect was seen at 120 minutes in both groups.

Effect of Methanol Fraction of *Delphinium denudatum* Root on Tail Flick Test in Rats

The rats in the Group I/Normal control responded within cut-off time of six seconds in all time periods [Table/Fig-5]. The Group II/Standard control showed statistically significant increase in reaction time (Seconds) as (p<0.001) 5.86, 6.77, 8.31, 9.13, 7.81, 6.72 and (p<0.01) 4.96 at the interval (minutes) of 30, 60, 90, 120,150, 180 and 210 respectively. Methanol fraction in low (Group V/MFDD200) and high (Group VI/MFDD400) doses showed statistically significant increase in reaction time (Seconds) as 4.19 (p<0.05), 5.14 (p<0.001), 5.40 (p<0.001), 5.63 (p<0.001), 5.26 (p<0.01), 4.98 (p<0.001), 4.33 (p<0.05), and 4.22 (p<0.05), 5.10 (p<0.001), 5.51 (p<0.001), 6.19 (p<0.001), 5.72 (p<0.001), 5.21 (p<0.001) at the interval (minutes) of 30, 60, 90, 120, 150, 180, 210 respectively. It was further noticed that the pain threshold was higher in the high dose group (Group VI/MFDD400). The peak effect in both groups was seen at 120 minutes.

DISCUSSION

The present study reveals the dose-dependent significant analgesic activity of the extracts of *Delphinium denudatum* in both the test.

In Eddy's hot plate and Tail flick test, the standard group of pentazocine showed significant (p<0.001) increase in reaction time. Administration of ethanolic extract of *Delphinium denudatum* in low (300 mg/kg) and high (600 mg/kg) doses showed statistically significant increase in reaction time. Similarly, the low dose (200 mg/kg) and high dose (400 mg/kg) of methanol fraction showed a significant increase in reaction time. It was also noticed that reaction time was higher in the large dose group in both ethanolic and methanol fraction. Further in ethanolic extract and methanol fraction, the peak effect in both groups was observed at 120 minutes.

Delphinium species are known for their diverse C19 and C20 Diterpene alkaloids. The analgesic activities of C18- and C19-diterpenoid alkaloids have been extensively investigated since 1981, among which 3-acetycarnitine, lappaconitine and crassicauline A, have been reported to exhibit remarkable analgesic activities and have been developed to be analgesic drugs clinically used for the treatment of various pains in China [12,13].

Alkaloids are commonly found to have analgesic activities [14]. The result obtained in this work demonstrated a high analgesic activity at low and high dose of ethanolic extract and methanol fraction of *Delphinium denudatum.* These results are in accordance to a study mentioning that hydro alcoholic extract of the *Delphinium denudatum* root and *Amaranthus spinosus* leaves produced significant effect in all the models of analgesic activity in a dose-dependant manner [9]. In addition, further studies related to *Delphinium denudatum* products has also shown antioxidant activities [15], antibacterial activity [16], nephroprotective activities [17] and morphine de-addiction properties [18] in a dose-dependent manner.

Group	0 minute	30 minutes	60 minutes	90 minutes	120 minutes	150 minutes	180 minutes	210 minutes	240 minutes
Normal Control	4.12±0.01	4.11±0.0	4.12±0.0	4.12±0.01	4.13±0.01	4.12±0.01	4.12±0.01	4.12±0.01	4.10±0.0
Positive Control	4.10±0.02	5.92±0.3***	6.67±0.1***	8.15±0.1***	9.02±0.3***	7.79±0.2***	6.73±0.18***	5.01±0.1***	4.13±0.02
EEDD 300	4.12±0.01	4.23±0.1	5.12±.0.2***	5.43±0.04***	5.82±0.06***	5.50±0.08***	5.11±0.06**	4.30±0.07	4.11±0.01
EEDD 600	4.11±0.02	4.34±0.2	5.31±0.04***	5.75±0.09***	6.52±0.10***	5.83±0.08***	5.03±0.08***	4.46±0.07**	4.13±0.02

[Table/Fig-3]: Effect of ethanolic extract of *Delphinium denudatum* root on reaction time in rats using Tail flick test.
Reaction Time: Mean±SEM (n=6) sec. *p<0.05, **p<0.01,***p<0.001 as compared to normal control EEDD: Ethanolic extract of Delphinium denudatum. (300 and 600 mg/kg)

Groups	0 minute	30 minutes	60 minutes	90 minutes	120 minutes	150 minutes	180 minutes	210 minutes	240 minutes
Normal control	3.93±0.22	3.93±0.22	3.83±0.23	3.91±0.21	3.85±0.21	3.83±0.24	3.92±0.23	3.83±0.22	3.82±0.21
Positive control	3.84±0.06	5.95±0.3***	7.22±0.45***	8.39±0.37***	8.77±0.17***	7.46±0.21***	6.41±0.26***	4.67±0.35	3.86±0.07
MFDD 200	3.90±0.12	3.99±0.12	4.60±0.16	4.98±0.13**	5.87±0.11***	5.59±0.12***	5.00±0.07**	4.14±0.96	3.89±0.13
MFDD 400	3.94±0.07	4.23±0.13	5.32±0.12**	5.99±0.09***	6.54±0.19***	6.04±0.08***	5.07±0.14**	4.08±0.16	3.88±0.18

[Table/Fig-4]: Effect of *methanol* fraction of *Delphinium denudatum* root in rats on reaction time using Eddy's hot plate test.
Reaction Time: Mean±SEM (n=6) sec. *p<0.05, **p<0.01,***p<0.001 as compared to normal control. MFDD: Methanol Fraction of *Delphinium denudatum*. (200 and 400 mg/kg)

Groups	0 minute	30 minutes	60 minutes	90 minutes	120 minutes	150 minutes	180 minutes	210 minutes	240 minutes
Normal Control	4.05±0.06	4.02±0.05	4.03±0.06	4.07±0.05	4.01±0.04	4.00±0.05	4.05±0.06	4.04±0.06	4.03±0.06
Positive Control	4.04±0.04	5.86±0.05***	6.77±0.17***	8.31±0.21***	9.13±0.26***	7.81±0.26***	6.72±0.21***	4.96±0.15**	4.11±0.03
MFDD 200	4.10±0.36	4.19±0.33*	5.14±0.06***	5.40±0.86***	5.63±0.08***	5.26±0.09***	4.98±0.07***	4.33±0.06*	4.16±0.06
MFDD 400	4.08±0.37	4.22±0.32*	5.10±0.05***	5.51±0.10***	6.19±0.11***	5.72±0.14***	5.21±0.14***	4.25±0.08*	4.09±0.48

[Table/Fig-5]: Effect of *methanol* fraction of *Delphinium denudatum* root on reaction time in rats using Tail flick test in rats.
Reaction Time: Mean±SEM (n=6) sec. *p<0.05, **p<0.01, ***p<0.001 as compared to normal control. MFDD: Methanol Fraction of *Delphinium denudatum*. (200 and 400 mg/kg)

The antinociceptive (analgesic and local anaesthetic) activity of diterpenoid alkaloids is based on braking of impulse conductivity on different stages of passing through nociceptive pathways. They analyse electrophysiological mechanisms of diterpenoid alkaloids showed that the sodium channels of central and peripheral neurons are their main target sites. The investigated substances may be divided into following types: 1) Activators of voltage-gated Na^+-channels altering selectivity of ion pore due to binding to site 2 of Na^+-channel. The substance induced a neuronal block by permanent cell hyperpolarisation; 2) Blockade of neuronal voltage-gated Na^+-channels by interacting to BTX-sensitive site. The substance inhibit the fast inward Na^+-current by ion pore bridging; 3) Blockade of ligand-gated Na^+-channels (N-acetylcholine blockers) that destroyed cholinergic transmission and downstream Na^+-current. These alkaloids selectively interact with nAChRs [19].

The present study reveals the dose dependent significant analgesic activity of the extracts of *Delphinium denudatum* in both the test. The increase in reaction time in both Eddy's hot plate and Tail flick test may be due to blocked of potential-gated Na+-channels which cause inhibition of the fast intake Na+-current or may be due to blockade of N-acetylcholinoreceptors (nAChRs) of CNS.

LIMITATION

The major limitation of the study was that the exact mechanism of action of *Delphinium denudatum* for analgesic activity could not be explained.

CONCLUSION

Authors conclude that the test drug *Delphinium denudatum* root in its ethanolic extract and methanol fraction showed statistically significant increase in reaction time (seconds) on Eddy's hot plate and Tail flick test. The study reveals the dose-dependent significant analgesic activity of the extracts of *Delphinium denudatum* in both the test. Thus the present drug used in traditional medicine might give a solution as an alternative remedy for pain management.

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