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An experimental study of ethanolic extract and methanolic fraction of *Delphinium denudatum* Wall in morphine withdrawal syndrome

Imran Zaheer[1], Syed Ziaur Rahman*[2], Rahat Ali Khan[3], Mehtab Parveen[4]

1 *Department of Pharmacology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh-202002, India*
2 *Associate Professor, Department of Pharmacology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh-202002, India*
3 *Professor, Department of Pharmacology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh-202002, India*
4 *Associate Professor, Department of Chemistry F/O Science, Aligarh Muslim University, Aligarh-202002, India*

Abstract

Objective: To evaluate the role of ethanolic extract and methanolic fraction of the roots of Delphinium denudatum Wall. in morphine dependence. **Methods:** Wistar albino rats were made moderately and severely morphine dependent by administering morphine sulphate in a dose of 10 mg /kg (i.p.) for 4 days and by increasing doses of 10-100 mg/kg (i.p.) for 7 days twice daily, respectively. The signs of spontaneous abstinence syndrome in both studies were recorded 12 hours after the last dose of morphine for 30 minutes and quantified by the 'counted' and 'checked' signs. Ethanolic extract (EEDD) and methanolic fraction (MFDD) of Delphinium denudatum was administered p.o. in different regimen: (a) 300 mg/kg (p.o.) of ethanolic extract or 200 mg/kg (p.o.) of methanolic fraction along with morphine twice daily for 4 days in moderately and 7 days in severely induced morphine dependence group, (b) Single dose of ethanolic extract 600 mg/kg (p.o.) or methanolic fraction of 400 mg/kg (p.o.) 10 h after the last dose of morphine in both moderately and severely induced morphine dependence rats. **Result:** Administration of EEDD and MFDD orally in both morphine dependent groups caused significant reduction in scores of counted and checked signs of morphine abstinence syndrome as compared to morphine control group. The reduction was observed more in regimen 'b' as compared to regimen 'a'. **Conclusion:** EEDD and MFDD of Delphinium denudatum root significantly reduced the mean scores of various 'counted signs' and 'checked signs' of morphine withdrawal syndrome and could thus be proved to be an alternative remedy in morphine de-addiction.

Keywords: *Delphinium denudatum*, Morphine sulphate, Morphine withdrawal syndrome, Morphine dependence.

INTRODUCTION

Morphine addiction is worst affected socio-economic problem worldwide. It causes mild to severe dependence resulting into risky withdrawal and hence produces difficulties at the time of de-addiction. A number of therapeutic medicines have been described in indigenous system of medicine to overcome the addiction. *Delphinium denudatum* Wall of family Ranunculaceae is a medicinal herb used in Unani Medicine under the vernacular name *Jadwar*. The roots of *Jadwar* are reported 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.[1] Nevertheless, its use in opium addiction is mentioned in classical literature.[2,3] Earlier studies showed that ethanolic extract of *Delphinium denudatum* has potential to attenuate the withdrawal symptoms in morphine dependence. [4] The present study is done to validate the earlier research work and to screen additionally the effect of methanolic fraction of *Delphinium denudatum* Wall.

Materials and Methods

Plant Materials

Roots of *Delphinium denudatum* were obtained from the local market of Aligarh. These were identified and authenticated by Dr. (Mrs) Sunita Garg, Chief Scientist, Raw Material Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi.

***Corresponding author:**
Dr. Imran Zaheer
Department of Pharmacology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh-202002, India

A sample specimen of plant material was deposited in the NISCAIR bearing voucher numbers "NISCAIR/RHMD/2013/2345-125-1".

Preparation of Extract

Delphinium denudatum roots were shade-dried. 100 g plant material was powdered with the help of an electrical grinder (REMI auto-mix blender, Vasai, India). For the preparation of ethanolic extracts, powdered roots were extracted with 300 ml of absolute alcohol with the help of Soxhlet apparatus. The extract was then filtered, collected and evaporated till it became dry at 40˚C on a water bath. The semisolid mass so obtained was weighed to calculate its yield in percentage. The yield of extract was 4.4%.

Preparation of Fraction

Delphinium denudatum roots were dried under shade and crushed to make powder of 1 kg. Air-dried powdered were exhaustively extracted with 95% ethanol. The solvent was removed by steam distillation and extract was concentrated to dark gummy mass under reduced pressure. The residue so obtained was fractionated by refluxing in the consecutive order with benzene, ethyl acetate and methanol. The yield of methanol fraction was 3.2%.

Animals Used

Wistar albino rats weighing 150-200 g of either sex were used in the study. The animals were procured from the Central Animal House of Jawaharlal Nehru Medical College, Aligarh. They were housed in polypropylene cages bedded with husk in the Pharmacology Section of the Central Animal House and provided with standard pellet diet (Ashirwad Industries, Chandigarh) and water *ad libitum*. The animal room was well-ventilated and maintained under standard environmental conditions throughout the experiment (temperature 18-29^{o}C, humidity 30-70%, 12 hour light/dark cycle). They were acclimatized to the laboratory condition for 1 week prior to experimental use. The study followed ARRIVE guidelines and was approved by the JNMC Animal Ethics Committee.

Acute Toxicity Study

No toxicity study was done for ethanolic extract of *Delphinium denudatum* roots because the drug had already been reported safe. However, toxicity study was done for methanol fraction of *Delphinium denudatum* roots on healthy adult female rats (100-150g) as per Organization for Economic Cooperation and Development (OECD) Guidelines 425. 2 g/kg each of methanol fraction of *Delphinium denudatum* was tested on five animals. All these animals survived at the end of 14 days of observation. Accordingly, the LD_{50} of each fraction is greater than 2000 mg/kg.

Drugs

Morphine sulphate (Inj. Morphitroy*15, Troikaa Pharmaceuticals Ltd., India).

DE-ADDICTION STUDY

Moderately induced Morphine dependence

Morphine in dose of 10 mg /kg (i.p.), twice daily for 4 days caused development of moderate morphine dependence.[4] Rats were divided into seven groups of five animals each. Group I received Propylene glycol 0.3 ml/100g p.o. twice daily for 4 days served as normal control (vehicle control) , Group II received Morphine Sulphate 10 mg/kg (i.p.) twice daily for 4 days served as Morphine control (Active control), Group III received ethanolic extract of *Delphinium denudatum* (EEDD) 300 mg/kg p.o. twice daily for 4 days served as drug control (positive control), Group IV received morphine sulphate 10 mg/kg (i.p.) along with ethanolic extract of *Delphinium denudatum* (EEDD) 300 mg/kg p.o. twice daily for 4 days, Group V received morphine sulphate 10 mg/kg (i.p.) twice daily for 4 days and ethanolic extract of *Delphinium denudatum* (EEDD) 600mg/kg single dose p.o. 10 hour after last dose of morphine, Group VI received morphine sulphate 10 mg/kg (i.p.) along with methanol fraction of *Delphinium denudatum* (MFDD) 200 mg/kg p.o. twice daily for 4 days, Group VII received Morphine Sulphate 10 mg/kg (i.p.) twice daily for 4 days and methanol fraction of *Delphinium denudatum* (MFDD) 400mg/kg single dose p.o. 10 hour after last dose of morphine.

The morphine abstinence syndrome, on which the assessment of physical dependence of morphine was based, consisted of a variety of motor and vegetative signs. The signs of spontaneous abstinence syndrome were recorded 12 hours after the last dose of morphine for 30 minutes. Similar observations were also done in drug control and normal control groups. The 'counted signs' and 'checked signs' were multiplied with the respective 'weighing factors' for evaluation of the severity of abstinence syndrome using a modified methods of Blasig J, et al. and Neil & Sparber [Table I].[5, 6]

Table I: Signs observed in rats 12 hours after the last dose of morphine for 30 minutes

Counted Signs	Weighing Factors	Checked Signs	Weighing Factors
Chewing	2	Scream on touch	1
Head Shake	2	Hostility on Handling	1
Exploring	1	Diarrhea	1
Digging	2	Eye Twitching	2
Yawning	2	Lacrimation	3
Teeth Chattering	2	Ptosis	2
Jumping	2	-	-
Wet Dog Shaking	2	-	-

Severely- induced Morphine dependence:

Morphine in increasing doses of 10-100 mg/kg (i.p.) twice daily for seven days is reported to cause development of severe dependence (Table II). The withdrawal signs were observed 12 hours after the last dose of morphine injection for 30 minutes in all groups.[4] The 'counted signs' and 'checked signs' were multiplied with the respective 'weighing factors' for evaluation of the severity of abstinence syndrome as done for moderately induced physical dependence.

Table II: Dose schedule of morphine in severely induced morphine dependence

Days	Time	Dose (mg/kg)
1st	12.00 Noon	10
2nd	12.00 Noon and 10.00 p.m.	10 and 20
3rd	12.00 Noon and 10.00 p.m	20 and 40
4th	12.00 Noon and 10.00 p.m	40 and 60
5th	12.00 Noon and 10.00 p.m	60 and 80
6th	12.00 Noon and 10.00 p.m	80and 100
7th	12.00 Noon and 10.00 p.m	100 and 100

Statistical Analysis

Data is represented as Mean ± SEM and analysed using one-way ANOVA followed by Tukey multiple comparison tests. P<0.05 were considered statistically significant.

RESULTS

Effect of ethanolic extract of *Delphinium denudatum* on parameters of abstinence syndrome in moderately-induced morphine dependent rats

The mean score of counted and checked signs in morphine group (study group III) were significantly increased (p<0.001) as compared to normal control (Table III).

Administration of EEDD in dose of 300 mg/kg orally twice a day for 4 days did not produce any sign of physical dependence (study group II). There was no difference in the counted and checked signs in D.d group from normal control group.

Administration of EEDD in dose of 300 mg/kg orally along with morphine 10 mg/kg (i.p.) twice daily for 4 days caused significant reduction in scores of counted and checked signs of morphine abstinence syndrome as compared to morphine control group observed 12 hours after the last dose of morphine (study group IV).

Mean score of counted signs such as chewing, headshakes, yawning, digging and teeth chattering in study group 4 were significantly decreased. Similarly, mean score of checked signs such as scream on touch was significant reduced. However, no significant change was observed in hostility on handling compared to morphine control group (Table III).

In group V, mean score of various counted and checked signs of morphine abstinence syndrome were markedly reduced as compared to group IV except chewing and headshakes. Comparative to group III, the mean score of counted and checked signs such as chewing, headshakes, yawning, digging, teeth chattering and scream on touch were significantly decreased. However, no significant change was observed in hostility on handling compared to morphine control group [Table III].

Table III: Effect of ethanolic extract of *Delphinium denudatum* on parameters of abstinence syndrome in moderately-induced morphine dependent rats

Groups	Chewing	Head shake	Yawning	Digging	Teeth Chattering	Scream on touch	Hostility On hand
Group I	1.2±0.4	0.0±0.0	1.2±0.8	2.0±0.8	0.0±0.0	0.0±0.0	0.0±0.0
Group II	1.2±0.4	0.4±0.4	1.2±0.8	2.4±1.1	0.0±0.0	0.6±0.2	0.2±0.2
Group III	11.6±1.1***	8.8±1.0***	14.8±1.0***	7.6±1.6***	10.4±1.3***	3.6±0.4***	1.6±0.7*
Group IV	4.8±1.0***	3.6±0.7***	4.8±1.0***	4.4±0.9*	6.4±1.1*	1.8±0.3**	0.8±0.3
Group V	6.0±1.0**	4.8±0.8**	2.4±0.7***	2.8±1.0**	0.8±0.4***	0.8±0.3***	0.6±0.4

Values are expressed as Mean ± SEM (n = 5) *P<0.05, **P<0.01, ***P<0.001. Comparisons between: Group III vs. Group I., And Group IV, Group V vs. Group III. Group I (Normal control), Group II (EEDD control), Group III (Morphine control), Group IV (EEDD300 test group), Group V (EEDD600 test group).

Effect of ethanolic extract of *Delphinium denudatum* on parameters of abstinence behaviour in severely-induced morphine dependent rats

Apart from chewing, head shakes, yawning, digging, teeth chattering, scream on touch and hostility on handling, which were observed during moderate abstinence syndrome, wet dog shakes, jumping, eye twitching and lacrimation were also observed in severely induced morphine dependent rats.

Mean score of counted and checked signs in morphine group (study group III) were significantly increased (p<0.001) compared to normal control (Table IV). These mean scores are more than the moderately induced morphine control group.

Administration of EEDD in dose of 300 mg/kg orally twice a day for 7 days did not produce any sign of physical dependence (study group II). Likewise, there was no difference in the counted and checked signs in EEDD group from normal control group.

Administration of EEDD in dose of 300 mg/kg orally along with morphine (in increasing doses 10-100 mg/kg) for seven days caused significant reduction in scores of counted and checked signs of morphine abstinence syndrome as compared to morphine control group observed 12 hours after the last dose of morphine (study group IV).

Mean score of counted signs such as chewing, headshakes, yawning, digging, teeth chattering, Jumping and Wet dog shake in study group IV were significantly decreased. Similarly, mean score on checked sign of scream on touch was significant reduced (P<0.001). However, no significant change was observed in hostility on handling, Eye twitching, Lacrimation compared to morphine control (Table IV).

In group V, mean score of various counted and checked signs was markedly reduced as compared to group IV except chewing and headshakes. Comparative to group III, the mean score of counted and checked signs such as chewing, headshakes, yawning, digging, teeth chattering, Jumping, wet dog shake and scream on touch, Hostility on handling, Eye twitching were significantly decreased. However, no significant change was observed in lacrimation compared to morphine control (Table IV).

Table IV: Effect of ethanolic extract of *Delphinium denudatum* on parameters of abstinence behaviour in severely morphine dependent rats

Parameter	Group I	Group II	Group III	Group VI	Group VII
Chewing	1.6±0.7	2.0±0.8	21.6±1.1***	12.8±1.0***	11.6±1.5***
Head shake	0.0±0.0	0.0±0.0	3.6±0.7***	1.20±0.4**	0.4±0.4***
Yawning	0.8±0.4	0.8±0.4	17.6±0.7***	11.6±0.7**	12.±1.6**
Digging	3.2±0.4	3.6±0.4	14.8±0.7***	10.4±0.7**	8.8±0.4***
Teeth chattering	0.0±0.0	0.0±0.0	19.2±1.0***	10.4±1.1***	8.8±0.8***
Scream on touch	0.0±0.0	0.0±.00	4.0±0.3***	1.6±0.4***	0.8±0.4***
Hostility on handling	0.0±0.0	0.0± 0.0	4.8±0.3***	3.4±0.6	2.2±0.5**
Wet dog shake	0.0±0.0	0.0±.0.0	2.8±0.4***	0.0±0.0***	0.0±0.0***
Jumping	0.0±0.0	0.0±.0.0	2.4±0.7***	0.0±0.0***	0.0±0.0***
Eye twitching	0.0±0.0	0.0±.0.0	4.4±0.7***	3.2±0.8	2.4±0.7**
Lacrimation	0.0±0.0	0.0±.0.0	3.6±0.6***	3.0±0.6	2.4±0.6

Values are expressed as Mean ± SEM (n = 5) *P<0.05, **P<0.01, ***P<0.001. Comparisons between: Group III vs. Group I., And Group IV, Group V vs. Group III. Group I (Normal control), Group II (EEDD control), Group III (Morphine control), Group IV (EEDD300 test group), Group V (EEDD600 test group).

Effect of methanolic fraction of *Delphinium denudatum* on parameters of abstinence syndrome in moderately-induced morphine dependent rats

Administration of MFDD in dose of 200 mg/kg orally twice a day for 4 day did not produce any sign of physical dependence (study group II). There was no difference in the counted and checked signs in D.d group from normal control group.

Administration of MFDD in dose of 200 mg/kg orally along with morphine 10 mg/kg (i.p.), twice daily for 4 days caused significant reduction in scores of counted and checked signs of morphine abstinence syndrome as compared to morphine control group observed 12 hours after the last dose of morphine (study group V).

Mean score of counted signs such as chewing, headshakes, yawning, digging and teeth chattering in study group 4 were significantly decreased. Similarly, mean score of checked signs such as scream on touch was significant reduced (P<0.001). However, no significant change was observed in hostility on handling compared to morphine control group (Table V).

In group VII, mean score of various counted and checked signs of morphine abstinence syndrome were markedly reduced as compared to group VI except chewing and headshakes. Comparative to group III, the mean score of counted and checked signs such as chewing, headshakes, yawning, digging, teeth chattering and scream on touch were significantly decreased (P<0.001). However, no significant change was observed in hostility on handling compared to morphine control group [Table V].

Table V: Effect of methanol fraction of *Delphinium denudatum* on parameters of abstinence syndrome in moderately-induced morphine dependent rats

Groups	Chewing	Head shake	Yawning	Digging	Teeth Chattering	Scream on touch	Hostility On hand
Group I	1.2±0.4	0.0±0.0	1.2±0.8	2.0±0.8	0.0±0.0	0.0±0.0	0.0±0.0
Group II	1.2±0.4	0.0±0.0	1.2±0.8	2.2±0.8	0.0±0.0	0.4±0.2	0.0±0.0
Group III	11.6±1.1***	8.8±1.0***	14.8±1.0***	7.6±1.6***	10.4±1.3***	3.6±0.4***	1.6±0.7*
Group VI	5.2±0.9***	4.8±0.6***	5.4±1.1***	4.9±1.0*	6.2±0.8*	2.0±0.3*	1.2±0.3
Group VII	5.0±1.0***	4.2±0.8***	3.6±0.8***	3.2±0.8***	1.0±0.7***	1.1±0.3***	0.8±0.4

Values are expressed as Mean ± SEM (n = 5) *P<0.05, **P<0.01, ***P<0.001. Comparisons between: Group III vs. Group I., And Group IV, Group V vs. Group III. Group I (Normal control), Group II (MFDD control), Group III (Morphine control), Group VI (MFDD200 test group), Group VII (MFDD400 test group).

Effect of methanolic fraction of *Delphinium denudatum* on parameters of abstinence behaviour in severely-induced morphine dependent rats

Administration of MFDD in dose of 200 mg/kg orally twice a day for 7 days did not produce any sign of physical dependence (study group II).

There was no difference in the counted and checked signs in D.d group from normal control group.

Administration of MFDD in dose of 200 mg/kg orally along with morphine (in increasing doses 10-100 mg/kg) for seven days caused significant reduction in scores of counted and checked signs of

morphine abstinence syndrome as compared to morphine control group observed 12 hours after the last dose of morphine (study group VI). Mean score of counted signs such as chewing, headshakes, yawning, digging, teeth chattering, jumping and wet dog shake in study group IV were significantly decreased. Similarly, mean score on checked sign of scream on touch was significant reduced (P<0.001). However, no significant change was observed in hostility on handling, eye twitching, lacrimation compared to morphine control (Table VI).

In group VII, mean score of various counted and checked signs was markedly reduced as compared to group VI. .Comparative to group III, the mean score of counted and checked signs such as chewing, headshakes, yawning, digging, teeth chattering, jumping, wet dog shake and scream on touch, hostility on handling, eye twitching were significantly decreased. However, no significant change was observed in lacrimation compared to morphine control (Table VI).

Table VI: Effect of methanol fraction of *Delphinium denudatum* on parameters of abstinence syndrome in severely-induced morphine dependent rats

Parameter	Group I	Group II	Group III	Group VI	Group VII
Chewing	1.6±0.7	1.8±0.6	21.6±1.1***	13.4±1.1***	12.2±1.3***
Head shake	0.0±0.0	0.0±0.0	3.6±0.7***	1.8±0.4**	1.0±0.4***
Yawning	0.8±0.4	0.6±0.4	17.6±0.7***	12.7±0.6**	12.9±1.2***
Digging	3.2±0.4	2.6±0.6	14.8±0.7***	10.0±0.3**	9.2±0.8***
Teeth chattering	0.0±0.0	0.0±0.0	19.2±1.0***	11.9±0.1***	9.6±0.4***
Scream on touch	0.0±0.0	0.0±.00	4.0±0.3***	2.2±0.4***	1.4±0.2***
Hostility on handling	0.0±0.0	0.0± 0.0	4.8±0.3***	3.9±0.4	2.6±0.5**
Wet dog shake	0.0±0.0	0.0±.0.0	2.8±0.4***	0.0±0.0***	0.0±0.0***
Jumping	0.0±0.0	0.0±.0.0	2.4±0.7***	0.0±0.0***	0.0±0.0***
Eye twitching	0.0±0.0	0.0±.0.0	4.4±0.7***	3.1±0.4	2.8±0.8***
Lacrimation	0.0±0.0	0.0±.0.0	3.6±0.6***	3.1±0.4	3.0±0.8

Values are expressed as Mean ± SEM (n = 5) *P<0.05, **P<0.01, ***P<0.001. Comparisons between: Group III vs. Group I., And Group IV, Group V vs. Group III. Group I (Normal control), Group II (MFDD control), Group III (Morphine control), Group VI (MFDD200 test group), Group VII (MFDD400 test group).

DISCUSSION

The study was conducted to evaluate the ability of *Delphinium denudatum* to suppress the signs of withdrawal in morphine-dependent rats. In our set up, we could observe only seven counted signs, viz. chewing, head shakes, digging, yawning, teeth chattering, jumping and wet dog shakes and four checked signs, viz. Scream on touch, hostility on handling, eye twitching and lacrimation out of all signs mentioned in Table I.

Earlier studies also observed that some signs were more prominent with less dependence and disappear as the degree of dependence increases, while other signs appear.[7] Further, the intensity of tolerance and precipitated withdrawal in rats is a function of the dosage and the interval of administration of morphine.[7] The results obtained in present study indicate that morphine sulphate in doses 10 mg/kg for 4 days caused development of moderate dependence which was confirmed by observing withdrawal signs after 12 h of the last dose of morphine.

EEDD caused reduction in the severity of the abstinence syndrome both IV and V groups. The maximum reduction in withdrawal signs was observed in group V in which the ethanolic extract was given in single dose (600 mg/kg) 2 h prior to the time of observation or 10 h after the last dose of morphine. Mean score of counted and checked signs such as chewing, headshakes, yawning, digging, teeth chattering and scream on touch were significantly decreased (($p < 0.001$, $p < 0.01$). However, no significant change was observed in hostility on handling compared to morphine control group.

MFDD also caused reduction in the severity of the abstinence syndrome in both VI and VII groups. The maximum reduction in withdrawal signs was observed in group VII in which the methanol fraction was given in single dose (400 mg/kg) 2 h prior to the time of observation or 10 h after the last dose of morphine. Mean score of counted and checked signs such as chewing, headshakes, yawning, digging, teeth chattering and scream on touch were significantly decreased (($p < 0.001$). However, no significant change was observed in hostility on handling compared to morphine control group.

Administration of morphine in increasing doses caused development of severe dependence. Reduction in the severity of the abstinence syndrome was observed in both extract and fraction group which were treated with EEDD and MFDD along with morphine in different dose regimens. In EEDD and MFDD treated group maximum reduction in withdrawal signs was observed in group V and VII in which the EEDD and MFFDD was given in single dose (600 mg/kg and 400mg/kg) 2 h prior to the time of observation or 10 h after the last dose of morphine. The scores for chewing, headshakes, yawning, digging, teeth chattering, scream on touch, hostility on handling and eye twitching were significantly decreased ($p < 0.001$). Whereas the score of wet dog shakes and jumping fell to zero in both extract and fraction treated group. It was observed that group IV and VI where the EEDD and MFDD were given in multiple doses (300 mg/kg and 200mg/kg twice daily × 7 days), the reduction in withdrawal signs was less as compared to group V and VII respectively. However, no significant change was observed in lacrimation compared to morphine control in both extract and fraction. It was further observed that reduction in withdrawal signs was more in extract treated group as compared to fraction treated group.

From our observation both test drug appears to act on sites other than opioid receptors because it neither caused development of dependence as observed by absence of withdrawal signs in group II. However, the drug caused attenuation of withdrawal signs, which appears to act centrally independent to opioid receptors.

Luis Tuesta *et al*., 2011 evaluated that endogenous cholinergic transmission at nAChRs play key role in regulating the reinforcing properties of addictive drugs including psychomotor stimulants, opiates, alcohol and cannabinoids.[8]

Feng B *et al*., 2011 studied that nAChR subtypes, i.e., α(4) β(2) and α(7), may contribute to the reinstatement of morphine-induced conditioned place preference (CPP) by drug priming in mice. They found that disruption of α4β2 or α7 nAChR signaling through DHβE or MLA administration, respectively, blocked the morphine-induced place conditioning.[9]

Alkaloids commonly found in *Delphinium* are derivatives of the norditerpenoid lycoctonine. Among many alkaloids, deltaline is a 7, 8-methylenedioxylycoctonine-type (MDL) norditerpenoid alkaloid; whereas methyllycaconitine (MLA), nudicauline, 14-deacetylnudicauline (14-DN), and barbinine are lycoctonine derivatives esterified with *N*-(methylsuccinyl) anthranilic acid at C18 and designated collectively as *N*-(methylsuccinimido) anthranoyllycacotonine (MSAL)-type alkaloids. The *N*-(methylsuccinyl) anthranilic acid moiety appears to affect alkaloid toxicity and affinity for nAChR types because norditerpenoid alkaloids lacking this group are at least 100 times less potent than the MSAL-type alkaloids. All five alkaloids blocked neuromuscular transmission in a concentration-dependent manner.[10] The alkaloid methyllycaconitine is a potent and selective α7 sub-type selective nicotinic acetylcholine receptor antagonist. [11, 12]

Tucci SA et al., 2003 studied that intrahippocampal administration of a high dose of nicotine had an anxiogenic effect in the social interaction test that was reversed by co-administration of methyllycaconitine (MLA), which is an antagonist at alpha7 and alpha3 nAChR subunits.[12]

Alkaloid methyllycaconitine (MLA), which inhibited acetylcholine and anatoxin induced whole-cell currents in cultured fetal rat hippocampal neurons, which was concentration dependent, reversible and voltage independent.[13] The distribution of MLA, a non-diterpenoid alkaloid, binding sites in rat brain has been reported: high density in hippocampus and hypothalamus, low density in striatum and cerebellum.[14] Yum L et al., 1994 reported that MLA produce nearly complete functional blockade of nicotinic receptors located at Edinger Westphal neurons in chick They also studied that MLA binds most avidly to α-bungarotoxin site in brain.[15] It also inhibits κ- bungarotoxin sensitive nicotinic receptor in ciliary ganglion.[15]

The blocked of α7 nAChR receptors by the *Delphinium denudatum* in experimental treated rats may be the explanation of decrease score of various counted and checked signs which manifested during morphine withdrawal. The exact sites and mechanism of action of *Delphinium denudatum* needs further evaluation. The extract seems to have a rapid onset as evident from the results of group V. In the light of the above findings, it has been noted that ethanolic extract and methanol fraction of *Delphinium denudatum* root significantly reduces the mean scores of various 'counted signs' and 'checked signs' of morphine withdrawal syndrome and thus may prove to be an alternative remedy in morphine de-addiction.

CONCLUSION

The ethanolic extract and methanolic fraction of the roots of *Delphinium denudatum* Wall significantly reduced the mean scores of various 'counted signs' and 'checked signs' observed as an inherent constituent of morphine withdrawal syndrome. The present test drug used in traditional system of medicine might give a solution as an alternative remedy in morphine de-addiction.

Conflicts of Interest

The authors declare no conflict of interest.

REFERENCES

1. Qudsia N, Jafri MA. Unani drug, Jadwar (Delphinium denudatum Wall.)-A review. Ind. J. Traditional Knowledge. 2006; 5(4): 463-67.
2. Ghani K N. Qarabadin-i Najmul Ghani (Urdu). 2nd ed. Lucknow: Naval Kishore Press; 1928, 62.
3. Shirazi Imaduddin. Treatise on Opium (Persian). Aligarh: Zillur Rahman Library, Ibn Sina Academy of Medieval Medicine and Sciences. circa 16th century manuscript.
4. Rahman S, Khan RA, Kumar A. Experimental study of the morphine deaddiction properties of *Delphinium denudatum* Wall. BMC Compl Alt Med, 2002; 2:6.
5. Blasig J, Herz A, Reinhold K, Zieglgansberger S. Development of physical dependence on morphine in respect to time and dosage and quantification of the precipitated withdrawal syndrome in rats. Psychopharmacologia (Berl.) 1973; 33:19-38.
6. Neil BS, Sparber SB. Mianserin attenuates naloxone-precipitated withdrawal signs in rat acutely or chronically dependent upon morphine. Pharmacol Exp Thera 1986; 236(1):157-65.
7. Tuesta L, Fowler CD, Kenny PJ. Recent advances in understanding nicotinic receptor signaling mechanisms that regulate drug self-administration behaviour. Biochem Pharmacol. 2011; 82(8): 984–95.
8. Feng B, Xing JH, Jia D, Liu SB, Guo HJ, Li XQ, et al. Blocking α4β2 and α7 nicotinic acetylcholine receptors inhibits the reinstatement of morphine-induced CPP by drug priming in mice. Behav Brain Res. 2011; 220(1):100-5.
9. Dobelis P, Madl JE, Pfister JA, Manners GD, Walrond JP: Effects of *Delphinium* Alkaloids on Neuromuscular Transmission. Pharmacol Exp Thera 1999; 291(2): 538–46.
10. Goodall KJ, Barker D, Brimble MA: A Review of Advances in the Synthesis of Analogues of the Delphinium Alkaloid Methyllycaconitine. Synlett 2005; (12): 1809-27.
11. Cheney DL, Goldstein A: Tolerance to opioid narcotics: time course and reversibility of physical dependence in mice. Nature (Lond.) 1971; 232:471-78.
12. Tucci SA, Genn RF, File SE: Methyllycaconitine (MLA) blocks the nicotine evoked anxiogenic effect and 5-HT release in the dorsal hippocampus: possible role of alpha7 receptors. Neuropharmacology 2003; 44(3):367-73.
13. Alkondon M, Pereira EF, Wonnacott S, Albuquerque EX: Blockade of nicotinic currents in hippocampal neurons defines methyllycaconitine as a potent and specific receptor antagonist. Mol Pharmacol 1992; 41:802-8.
14. Davies AR, Hardick DJ, Blagbrough IS, Potter BV, Wolstenholme AJ, Wonnacott S.Characterization of the binding of [3H] methyllycaconitine: a new radioligand for labeling alpha 7-type neuronal nicotinic acetylcholine receptors. Neuropharmacol 1999; 38:679-90.
15. Yum L, Wolf KM, Chiappinelli VA: Nicotinic acetylcholine receptors in separate brain regions exhibit different affinities for methyllycaconitine. Neuroscience 1996; 72:545-55.