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Original Article

Screening of Analgesic Activity of *Delphinium Denudatum* and *Amaranthus Spinosus* in Experimental Animals

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ABSTRACT

Objective: Screening of analgesic activity of *Delphinium denudatum* and *Amaranthus Spinosus* in experimental animals.
Methods: In the present study to investigate the effect of the hydro alcoholic extract of the *Dephinium denudetum* root and *Amaranthus spinoses* leaves for analgesic activity.Analgesic activity determined by reduction in the number of writhing and increased in the reaction time with the animals.
Results: Hydro alcoholic extract of the *Dephinium denudetum* root and *Amaranthus spinoses* leavesproduced significant effect in all the models of analgesic activity in a dose dependant manner.
Conclusion: Hydroalcohlic extracts of *Dephinium denudetum* root and*Amaranthus spinoses*leaves and combination of both the drugs may act as an analgesic agent in rats. *Amaranthus spinoses*was found to be more effective compare to the *Dephinium denudetum.*
***Key words*:** Analgesic, *Delphenium Denudetum, Amaranthus spinosus,* Pain etc.

1. INTRODUCTION

Pain as an obnoxious sensory and emotional incident allied with actual or potential tissue damage[1].Pain is associated with a number of diseases. Drugs that are currently used for the management of painare opioids or nonopioids and that for inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. All these drugs carry potential toxic effects. It was reported that the risk of gastrointestinal bleeding was significantly associated with acute use of non-steroidal anti-inflammatory

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drugs(NSAIDs) like regular-dose aspirin, diclofenac, ketorolac,naproxen or nimesulide and Piroxicam etc., increased the risk of bleeding in both acute and chronic therapy[2]. There is an estimated market only of analgesics is $ 20 billion and approximately $ 4 billion needed to treat their side effects. Statistics from The Daily Telegraph suggests that 2000 deaths per annum are due to aspirin alone due to high risk of side effects like blood dyscrasia, nervous disorders, vomiting, nausea,bleeding tendency, metabolic disorders and rashes[3]. In contrast, plant origin drugs having very less undesirable effect and represent a large natural source of useful compounds that might serve as lead for the development of novel drugs effects. The drugs of plant origin are gaining increasing popularity and are being investigated for remedies of a number of disorders[4,5].

The *Delphinium denudatum*(Jadwar) belongs to the family *Ranunculaceae,* which is employed for a number of Indian systems of medicines for different types of disorders and diseases[6].Jadwar reported to have some pharmacological activities like antibacterial, antifungal, anticonvulsions, hepato-protective activities and deaddiction of morphine etc[7]. *Amaranthus spinosus* is an erect plant, found in different color in different region of Asia like Japan, Indonesia and India, etc. In India, it is reported that the plant having a number of medicinal properties like a laxative, diuretics, antipyretics, antibronchitis, anticonvulsion and wound healing etc[8, 9] . There is a lack of scientific data related to analgesic activity for these two plants. In this context, the present study is designed to carry out comparative and combined study of *Dephinium denudetum* root and *Amaranthus spinoses* leaves for analgesic activity.

2. MATERIAL AND METHODS

Al chemicals and reagents used for this study were of analytical grade and procured from approved organization.

Preparation of Extracts

The plant material root of *Delphenium denudatum* was collected from local market of Moradabad, Uttar Pradesh and leaves of *Amaranthus spinosus* from IFTM university botanical garden. The parts of plants used were recognized and legitimated by the Botanist, Dr. Beena Kumari, Hindu College, and Dr. Ashok Kumar IFTM University, Moradabad Uttarpradesh. The botanical nomenclature of the *Delphenium denudetum* and *Amaranthus spinoses* The botanical nomenclature of the plants was suitably recognized by matching with herbarium records and standard flora. The parts of the plants were dried under the shade and crushed into the coarse powder and passed through a 20-mesh sieve, then defatted with petroleum ether (60-80 ^{0}C).

After this, defatted markedly extracted with hydroalcohlic solvent (Ethanol 95%, v/v: water, 1:1) in soxhlet apparatus. The extracts were filtered and concentrated separately by removing the solvents up to the dryness using a rotatory vacuum evaporator.

Animals:

The recent experimental work was done on either sex of Wistar albino rats (Dinesh et al., 2012, Baba et al., 2016, Gireesh et al., 2013) weighing (150-200g). Animals were procured from the animal house of the I.F.T.M. University, Moradabad and maintained on a natural day–night cycle (12hr dark: 12hrs light) at room temperature of about 24-26°C, with free access to standard food pellets and water *ad libitum*. Before exposure to experiment, animals were acclimatized for at least ten days. Experiments were carried out between 10:00am-5:00pm. The study was approved by the Institute Animal Ethics Committee, Department of Pharmacology and Clinical Research, College of Pharmacy, IFTM University, Moradabad. The animals were divided into 7 groups and each group contained six animals (n=6).

2.4. Acute toxicity studies:

The test drugs administered in the range of the doses (50–2000 mg/kg, p.o.) *of* roots *Dephinium denudetum* extract (DDE) and leaves *Amaranthus spinoses* extract (ASE) to the animals and continuously observed for one and a half hourly intervals for 4 hrs, for any gross behavioural changes upto the 72 hrs followed by 14 days for any mortality[10].

'Experimental studies

Analgesic activity

Writhing tests (WT):

Animals were treated with the test and standard drugs for 7 successive days once a day and test was performed on the 7th day after 60 min administration of test drugs per oral and 30 min after standard drugs (Diclofenac sodium 6 mg/kg, i.p.) administration by i.p.

Grouping and treatment of animals is as follows

Group I	Control group –Vehicle (2 % Tween 80 in distilled water (5ml/kg,po)
Group II	Received DDE (200 mg/kg, p.o.)
Group III	Received DDE (400 mg/kg, p.o.)
Group IV	Received ASE (200 mg/kg, p.o.)
Group V	Received ASE (400 mg/kg, p.o.)
Group VI	Combi. C (100 mg/kg, p.o.)
Group VII	Receive standard drugs (Diclofenac 6 mg/kg, p.o.)

The writhing test was performed as described by Koster[11]**.** After 30 min of treatment, each rate of each group was administered intraperitoneally with 0.6% acetic acid in normal saline at the dose 10 ml/kg. The rats were observed and counted for the number of abdominal constrictions and stretching's in a period of 0-20 min. A reduction in the writhing number compared to the control group was evaluated for analgesia activity which was expressed as % inhibition of writing's.

Hot plate tests:

The Hot plate test used to evaluate the thermal pain reflexes due to foot pad contact with a heated surface. Rats of either sex weighing 130-140 gm. were divided into 8 groups of six animals each. The animals were placed on the hot plate maintained at constant temperature of 55^{0}C and reaction

time (in seconds) jumping of paw responses was noted. This was repeated at 60, 120, 180 minute time of intervals. A cut off period of 15 sec was observed to avoid damage to the paw[12]. Treatment and groups remain same as WT.

Tail Immersion test

Prior to the analgesic experiments, the animals were grouping screened for a sensitivity test by immersing the tip of tail (5 cm) gently in hot water (550ºC).Within a few seconds, the rats react by withdrawing tail. The reaction time is recorded by stopwatch. The reaction time was determined periodically after administration of the drugs. The cut off time of tail immersion was taken 15 seconds[13]. Treatment and grouping remain same as WT.

3. RESULTS

In this test, it was observed that DDE and ASE (200 and 400 mg/kg),Combi. C (100 mg/kg) and Diclofenac 6mg/kg reduced significantly the number of writhing compared to the control as the results are seen in table 1.

Table 1: Effects of DDE, ASE and combination of both the drugs in writhing test.

S.N0	Treatment groups (mg/kg, p.o)	Number of writhing's	Inhibition (%)
1	Control (5 ml)	17.83±2.66	0
2	DDE 200	14.33±2.31*	11.77
3	DDE 400	14.01±0.36*	17.32
4	ASE 200	13.45±1.34*	11.23
5	ASE 400	13.32±3.23*	23.53
6	Combi. C 100	12.45±2.08*	29.42
7	Diclofenac 6	12.01±3.42**	41.18

All values are expressed as Mean ± SEM, test employed ANOVA one way followed by Dunett's test (n=6); significant different from the control at *(P<0.05), ** (P<0.01), ***(P<0.001) when compared to the control group.

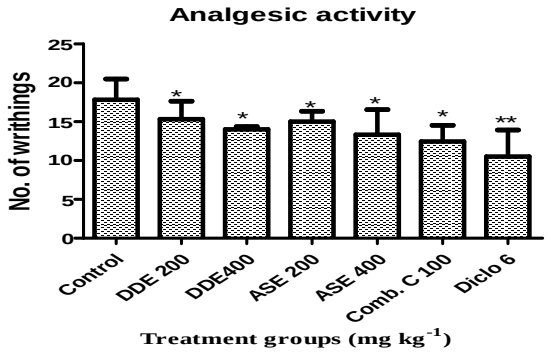

Fig 1: Reflecting the effects of DDE, ASE and combination of both the drugs in writhing test.

In this test, it was found that DDE and ASE produced significant effect in dose dependant manner after 60 and 120 min after administration. ASE, combination of both the drugs and standard drug increased the reaction time significantly till 180 min when compared with the control as the results are seen in the table 2

Table 2: Effects of DDE, ASE and combination of both the drugs in tail flick method.

S.N.	Treatment groups (mg/kg, p.o.)	Reaction time in sec		
		After 60 min	After 120 min	After 180 min
1	Control (5 ml)	2.16±0.32	2.33±0.33	2.33±0.33
2	DDE 200	3.98±0.32*	3.83±0.63*	2.83±0.30
3	DDE 400	5.66±0.42***	4.30±0.36**	2.66±0.21
4	ASE 200	4.33±0.42**	4.66±0.33**	2.83±0.30
5	ASE 400	5.00±0.36***	5.16±0.30***	4.00±0.36*
6	Comb. C 100	6.83 ±0.60***	5.16±0.70***	6.86±0.47**
7	Diclofenac 6	7.66±0.66***	10.83±0.30***	10.17±0.79***

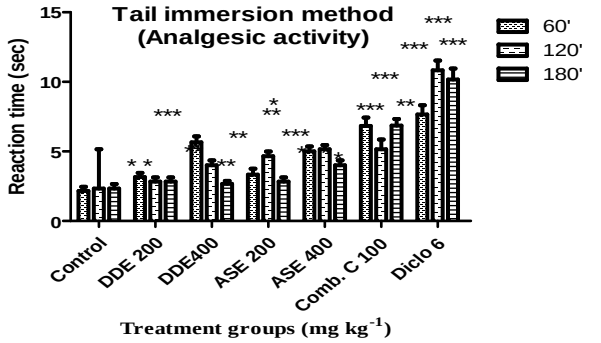

Fig 2: Showing the effects of DDE, ASE and combination of both the drugs in tail immersion test.

In this test, it was found that DDE (200 mg/kg) and ASE (200 mg/kg) produced significant (p<0.05) effect after 60 min and more significant (p<0.01) effect only after 120 min, ASE and DDE (400 mg/kg) showed more significant effect (p<0.01) after 60 and 120 min, where as Combi. C2 and standard drug more significantly (p<0.01) increased the reaction time at 60, 120 and 180 min when compared with the control as the results are seen in the table 3.

Table 3: Effects of DDE, ASE and combination of both the drugs in hot plate test.

S.N.	Treatment groups (mg/kg, p.o.)	Reaction time in sec		
		After 60 min	After 120 min	After 180 min
1	Control (5 ml)	2.66±0.42	2.65±0.42	2.65±0.42
2	DDE 200	4.66±0.66*	4.50±0.61	2.67±0.33
3	DDE 400	6.00±0.93***	5.50±0.61**	4.66±0.36*
4	ASE 200	5.16±0.25**	5.00±0.30*	2.66±0.33
5	ASE 400	6.00±0.44***	5.83±0.30***	4.83±0.47*
6	Comb. C 100	9.66 ±0.66***	9.66±0.55***	5.20±0.31*
7	Diclofenac 6	11.17±0.30***	11.17±0.30***	9.83±0.65***

All values are expressed as Mean ± SEM, test employed ANOVA one way followed by Dunett's test (n=6); significant different from the control at *(P<0.05), ** (P<0.01), ***(P<0.001) when compared to the control group.

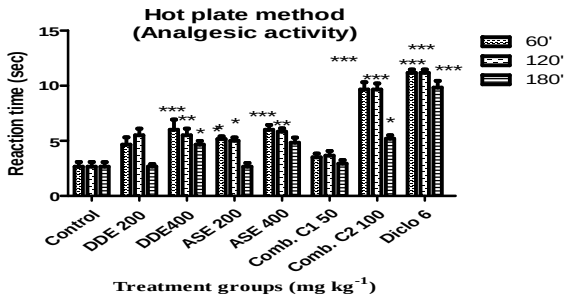

Fig 3: Reflectingtheeffects of DDE, ASE and combination of both the drugs in hot plate method.

4. DISCUSSION

The results in this study indicated that tested doses markedly exhibited a dose-related analgesic activity. The potency of

the test drugs could be compared to a reference standard Diclofenac. The experimental animal models used in this study are sufficient for evaluation of analgesic mechanisms of the extract both peripherally and centrally mediated effects. The acetic acid tests (chemical stimuli) are used to elucidate the peripheral and central mediated action, while hot plate and tail flick tests (thermal stimuli) are applied for investigation of the central mediated mechanism. The test drugs clearly demonstrated analgesic activity in all experimental animal models used in this study. Therefore, these results could be implied that the test drugs would have analgesic mechanisms mostly centrally mediated[11].

Pain is 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage [14].The Pain may fell because of inflammation, infection, ischemia, tissue necrosis, chemical or burn. In the stomach and intestines, pain may result from inflammation of the mucosa of from distension or muscle spasm. Depending of the cause, pain may be sudden and short-term marked primary by reflex withdrawal[15]. When an injury occurs, pain is first evoked by stimulation of the nociception (Aδ fibber's and C fibres) causing potassium and kinins to be released from the damaged cells. These stimulate the receptor directly resulting in the release of the neuropeptides such as substance P from nociceptive terminals and the release of the histamine from the mast cells in the production of the platelet-activating factor (PAF) which in turn releases serotonin from the platelets. Histamine is also released from the mast cells, starting an inflammatory reaction leading to vasodilation and edema[16]. Bradykinin is released upon tissue injury. Bradykinin is an important substance to stimulate nociception and prostaglandins sensitize nociception sending impulses to the spinal cord. This state is called peripheral sensitization. When signal transduction comes to post central gurus in the thalamus, which is responsible for the conscious perception of pain. This state is called central sensitization[17].

In the present study, results indicated that the HAEGPR, HAEGPL showed an analgesic effect against pain induced by experimental animal models included chemical stimuli in the acetic acid-induced writhing and the thermal stimuli in the hot plate and tail flick tests is used to screen both peripherally and centrally acting analgesic activity[18]. The writhing test can predict effective analgesic doses of agents that can be used in humans[19].Acetic acid cause pain by liberating endogenous substance, including serotonin, histamine, prostaglandins, bradykinin and substance P and many others that excite pain nerve ending leading to the abdominal writhing[20].The writhing response of the animal to an injection of noxious chemical is not very specific nociceptive model, it is important to reveal a general anti-nociceptive effect of the extract under study[21].The analgesic action of non-steroidal anti-inflammatory drugs (NSAIDs) is exerted both peripherally and centrally. In this study, the reference drug Diclofenac (6 mg/kg) more significantly decreased the number of writhing's also exhibited significant protective effects towards the acetic acid -induced pain in rats. In comparison, the analgesic potency of the DDE, ASE (200 and 400 mg/kg) and a combination of the drugs could be comparable with Diclofenac in rates. Diclofenac is usually associated with anti-inflammatory action and results from the inhibition of prostaglandin synthesis via cyclooxygenase. Prostaglandin's produced little pain by themselves, but potentiates the pain caused by other mediators (such as histamine and bradykinin)[22].

Pain induced by thermal stimuli included hot plate and tail flick tests is known to be selective to centrally but not peripherally acting analgesics[23] . The hot plate and tail immersion tests are considered specific tests for evaluation of the central pain at the supraspinal and spinal levels[21]. The hot plate method is one of the most common tests used for evaluating the analgesic efficacy of drugs in rodents[24]. The drug that reduces the nociceptive response indicated by cutaneous thermic stimuli in the hot plate test might exhibit central analgesic properties or supraspinal analgesia[25].Thermic painful stimuli are known to be selective to centrally, but not peripherally acting analgesic drugs [23].The results reflected that the test drugs and combination of both the drugs (higher doses) produced the good analgesic effect, but it was as not so long as produced by the standard drug. The test drugs may have good onset of action but the duration of action was found to be short.

All results obtained from writhing, hot plate and tail immersion tests used in this study indicated that the both the test drugs may possess centrally mediated analgesic activity.

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