Iranian Journal of Pharmaceutical Research (2004) 4: 231-236
Received: June 2004
Accepted: August 2004

Original Article

Antinociceptive effects of oral and intraperitoneal administration of alcoholic *Datura stramonium* seeds extract in male rats

Mohsen Khalili* and Masoud Atyabi

Department of Physiology, Shahed University of medical Science, Tehran, Iran.

Abstract

The present study is a designed protocol for investigation of the analgesic effect of oral and intraperitoneal (i.p.) administration of alcoholic *Datura stramonium* (DS) seed extract as a rich source of alkaloid substances. Male NMRI rats were divided into control and treatment groups. The treatment rats received different doses of the DS seed extract, which was prepared from alcoholic smashed seeds. Then, the animals from each group were subjected to pain scoring experiments such as hot plate and formalin tests. The results of the experiments i.e., antinociceptive effect of DS seeds extract in i.p (5, 10, 25, 50, 100, 200 and 250 mg/kg) and oral application methods (200, 400 and 800 mg/kg) were compared with other groups, morphine sulfate and naloxone as positive and negative control groups, respectively. We found the 30 and 100 mg/kg of the extract as intraperitoneal ED50 in the hot plate and formalin tests, respectively. However, the extract over than 100 mg/kg, i.p could potentially alleviate the pain in hot plate and both phases of formalin test. Besides, there was a marked antinociceptive effect for the extract (over than 400 mg/kg) in oral method in hot plate and both phases of formalin tests. In our following experiments the effective doses of morphine sulfate as positive control test were obtained over than 15 mg/kg; i.p. and the acquired LD50 was close to 2300 mg/kg.

In summary, comparing the analgesic effect of different doses of morphine sulfate with DS seed extract in i.p and oral conditions and considering to the extract LD50, we conclude that the DS seeds extract have a potent, absorbable, and nearly safe ingredient which can exert a potential analgesic effect in acute and chronic pain.

Key word: *Datura stramonium;* Formalin test; Hot plate; Rats.

Introduction

The medicinal plant *Datura stramoniom* (DS) (Fam. Solanaceae) has been introduced as an analgesic plant in Iranian folk medicine (1). Recently, it has been used as a narcotic and local anesthetic drug in many societies (2-4). Also, in some nations young people use its leaves by smoking for hallucination purpose (2). Some experiments have reported that DS extract is rich in alkaloids especially antimuscarinic components (5, 6). These alkaloids are more abundant in DS seeds than other parts of the plant (7).

* Corresponding author:
E-mail: najafabady@yahoo.com

It has also been shown that there is an interaction between opioid and cholinergic systems (8, 9). However, it is evident that the role of cholinergic system on the pain was mediated by its central effect on inhibitory opioid pathways (10, 11) through spinal cord and brain stem opioid receptors (12). Since the role of opioid receptors in antinociception, especially in acute pain has already been established, (e.g. interaction of these receptors with cholinergic system) and since the DS seed extract is known to be a rich source of anticholinergic gradients, this herb may be a good candidate for therapeutically analgesic purposes.

In addition, concerning to many side effects and insufficiency of common analgesic drugs and continuous recommendation of herbal medicines by the physicians (1), the present study was proposed to investigate the analgesic effect of DS seeds extract, using i.p and oral methods in rates.

Experimental

Preparation of crude extract

The seeds of medicinal plant *Datura stramonium* were obtained from the local market and scientifically identified by the department of botany of Shaheed Beheshti University (SBU). One hundred grams of cleaned DS seeds was crushed and mixed at a ratio of 1 to 4 with methyl alcohol. The mixed complex was set aside for 24 h in laboratory temperature. Then, it was filtered three times through a mesh. The alcohol of filtered solution was evaporated in a 50oC tissue organ bath. Finally, 8-12 g concentrated residue remained in the container, which was used for preparation of extract doses (mg/kg).

Animals

Male NMRI rats with 300-350 weight ranges (Razi Institue, Iran) were used in our experiments. Four animals were housed in each plexiglass cage with free access to food and water. The laboratory temperature and light-dark cycling was 24±2oC and 12 h, respectively.

Table 1. Effects of DS seed extract, morphine -sulfate and naloxone in hot plate test

		Hot plate latency (s)			
Treatment	Dose (mg/kg)	Pre-drug	Post-drug	P	*n*
Saline		17±0.82	18±0.91		10
Morphine	5	18±0.25	16±0.91		7
	10	19±0.33	14±0.11	*	9
	15	24±0.68	13±0.83	**	7
Naloxone	5	19±0.60	21±0.11		6
	10	21±0.14	19±0.19		7
	20	17±0.98	18±0.63		9
DS, i.p	5	22±0.98	20±0.88		7
	10	13±0.68	17±0.33	*	9
	25	13±0.78	20±0.16	*	12
	50	11±0.15	21±0.45	**	12
	100	09±0.32	18±0.26	**	12
DS, orally	200	23±0.62	21±0.78		8
	400	12±0.91	21±0.88	**	9
	800	12±0.28	19±0.20	**	9

Hot plate latency: Mean±SEM. * P<0.05, ** P<0.01

Antinociceptive tests

The DS seed extract in 5, 10, 25, 50, 100, 200 and 250 mg/kg, i.p. were given to the rats 15-20 min prior to measuring the pain. Also, in oral method, doses of 200, 400 and 800 mg/kg were prescribed to the animals via a gavage tube into the stomach, 40-50 min before the tests. In positive and negative control tests, morphine sulfate (5, 10 and 15 mg/kg; i.p.) and naloxone (5, 10 and 20 mg/kg; i.p.) were used, respectively. Then, in order to assess the pain, all of the animals were subjected to the hot plate and formalin tests. The saline injected rats were used as control group.

Hot plate test

Antinociception was assessed with a hot plate apparatus (Harvard-UK). The rats were acclimated in the turn-off hot plate apparatus before scoring the pain, 4-5 times with 5 min interval. The time between standing of the animals on the turned-on hot plate (54oC) till licking of burned paw was measured and considered as the pain score. Each animal was tested 5 times with 5 min intervals. The animal's paw was prevented from tissue injury, because in our experiments the duration of the test was not over than 30 s. In treatment group we calculated the percentage of Maximum Possible Effect (MPE) of the extract with following formula (13).

$$MPE = \frac{Test\ Latency(s) - Baseline}{Cutoff\ Time(s) - Baseline} \times 100$$

Where baseline and test latency are the pain threshold times before and after the extract application respectively, and the cut off time is the maximum time that the animals are permit to stay on the hot plate apparatus (30 s).

Formalin test

The method of formalin test introduced by Dubuisson and Dennis (1977) was used in our experiments. Formaldehyde (50 µl, 2.5%) was injected subcutaneously into the plantar

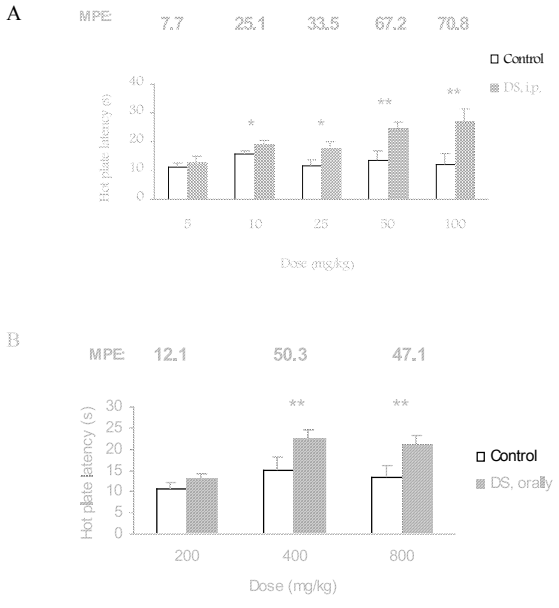

Figure 1. (A) Effects of intraperitonealy administration of the DS seed extract in the doses of 5, 10, 25, 50, 100 mg/kg on acute pain produced by the hot plate test. The extract could produce an analgesic effect dose-dependently with an ED50 close to 25 mg/kg. (B) Antinociceptive effects of the extract when administered through the mouth. A marked analgesic effect was found following the administration of the extract in the doses of 400 and 800 mg/kg. The Maximum Possible Effect (MPE) for each dose is represented. n=8-12 for each group, *P<0.05, **P<0.01.

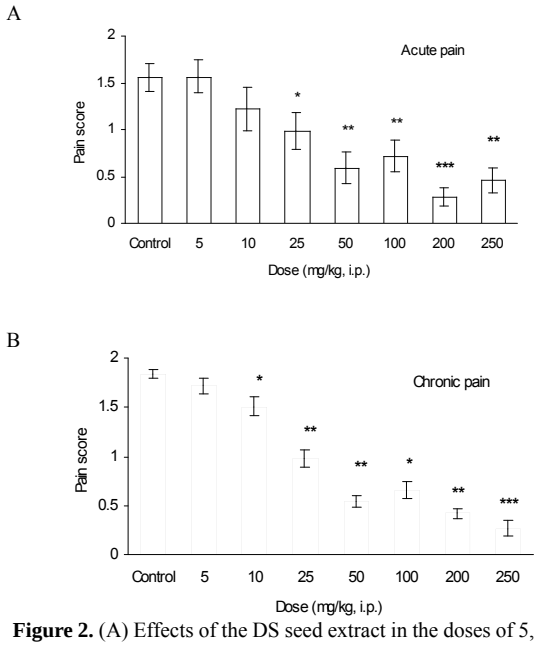

Figure 2. (A) Effects of the DS seed extract in the doses of 5, 10, 25, 50, 100, 200 and 250 mg/kg i.p. on acute phase of formalin test. The extract could produce antinociceptive effect dose-dependently, with maximum effect in 200 mg/kg. (B) Effects of 5, 10, 25, 50, 100, 200 and 250 mg/kg, ip of the extract on the second phase of formalin test. Although, the extract in 10 mg/kg diminished the pain markedly, but the maximum analgesic effect was elucidated in doses over than 200 mg/kg. n=12-14 for each group. * P<0.05, ** P<0.01. *** P<0.001.

surface of hind paw, and then the animal was placed in a plexiglass chamber (30×30×30 cm) which has a mirror with 45o angle underneath in order to accurate observation. In the treatment groups, the DS seed extract was administered intraperitoneally and orally, prior to the formaldehyde injection, 15-20 and 40-50 min, respectively. All animals were brought to the test chamber 5 times with 5 min intervals before the experiments in order to be adapted to the environment. The behavioral pain reactions due to formalin injection were detected and recorded for 1 h. The first 10 min, post-formalin injection is known as the early phase (acute phase), and the period between 15-60 min is as the late or chronic phase.

Statistical analysis

The result of each dose of the extract was expressed as mean±SEM. The differences were estimated by ANOVA and followed by Tukey's test. We considered the probability of P<0.05 as a significant difference.

Results and Discussion

Hot plate test

Intraperitoneal method

Figure 1.A shows the antinociceptive effect of different doses of DS seed extract in i.p. method in comparison to the control groups. Each dose was administered in 8-12 rats. As indicated, the dosage of 25 mg/kg is close to the ED50, and curve fitting calculations (LSD) show it near to 30 mg/kg. However, in contrast to the weak analgesic effect of the extract in the doses of 5 and 10 mg/kg, marked antinociceptive effect was obtained in the doses of 50 and 100 mg/kg.

Oral method

Administration of the extract in doses over than 400 mg/kg through the mouth could attenuate the acute pain, significantly (Fig. 1B).

In table 1, the analgesic effect of i.p and oral administration of the DS extract were compared with different doses of morphine sulfate and naloxone in the hot plate test. As shown, in

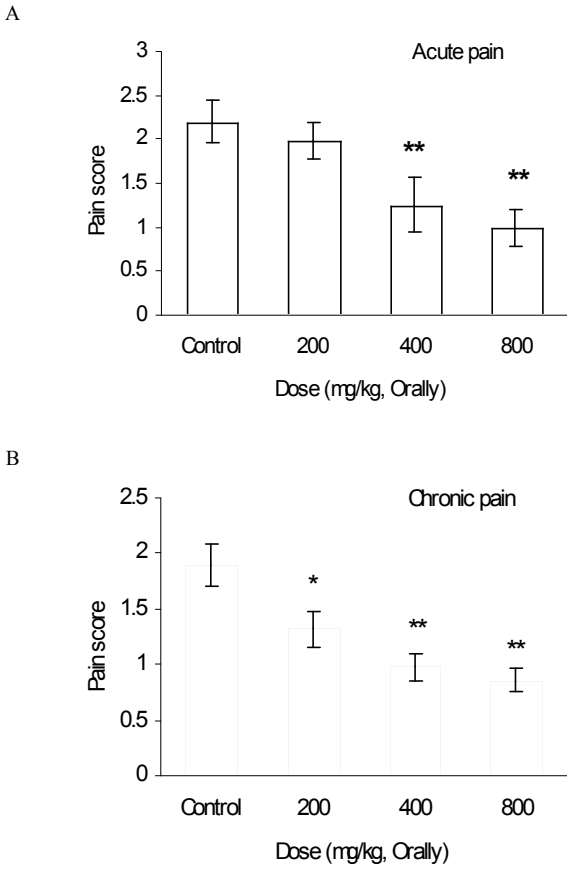

Figure 3. (A) Effects of oral administration of the DS seed extract in doses of 200, 400 and 800 mg/kg on acute phase of formalin test. The extract potentially alleviated the acute pain in the doses of 400 and 800 mg/kg. (B) Effects of different doses of the extract on the chronic phase of the formalin test. Except for moderate effect of the extract in 200 mg/kg, it could markedly diminish the pain score in the doses of 400 and 800 mg/kg. n=12-14 for each doses. * P<0.05, ** P<0.01.

contrast to the inefficacy of different applied naloxone doses in the relief of the acute pain, morphine sulfate in the doses of 10 and 15 mg/kg i.p; and the extract in both i.p (over than 10 mg/kg) and oral method (over than 400 mg/kg) alleviated the pain in a does-dependent manner.

Formalin test

Interaperitoneal method

Administration of the extract in the doses of 5 and 10 mg/kg did not produce a marked analgesic effect in acute (Fig. 2A) and chronic (Fig. 2B) formalin phases. However, doses of 25, 50 and 100 mg/kg showed mild to moderate (P<0.05 and 0.01) and higher doses over than 200 mg/kg showed profound analgesic effects in both first (A) and second (B) phases of formalin test (P<0.01 and 0.001). According to the results, antinociceptive effect of the extract is dose-dependent, and by curve fiting method the ED50 was found to be close to 100 mg/kg.

Oral method

In figure 3 the effect of different doses of the extract (200, 400 and 800 mg/kg) in oral route were compared with control test. The formalin pain score (as mean±SEM) in control, extract treatment and positive control groups (Morphine sulfate) were compared with others (Fig. 4). Application of the extract and morphine sulfate markedly diminished the acute formalin pain, at the chronic phase of the pain, especially its ultimate periods (30-60 min) was significantly attenuated by the extract.

Median lethal dose (LD50) test

The DS seed extract was injected i.p., in the doses of 500, 1000, 1500, 2000, 2500, 3000 and 4000 mg/kg to the animals in separated group (n=12-16). In our experiments the dosage of 2500 mg/kg nearly killed 55% of the animals 72 hours after the injection (Table 2). According to the graphical analysis (LSD) the LD50 was intimately obtained close to 2300 mg/kg.

Pain as a real complaint in clinical training, has different causing factors. Although there are many analgesic drugs for prescription, but because of many complexities including broad side effects, different origins of pain and weak potency of many conventional drugs (14, 15), medicinal plant substitution has been recommended for this purpose (16).

In the present study, we used alcoholic DS seed extract, because it contains a rich source of alkaloids in comparison to the other parts of the plant (3). Because, these alkaloids are mainly antimuscarinic components (4), they can probably interact with opioid system (8, 9). It could be resulted that the suggestive mechanism for DS analgesic effect was carried out via its alkaloids. The use of DS extract for local anesthesia in some nations (1, 17) and analgesic effect of some species of Datura like Fastuosa and Ceratocaula (3) are consistent with our report. In our study, the extract exerted the analgesic effect in both hot plate and formalin tests. Because the acute and chronic pain, were

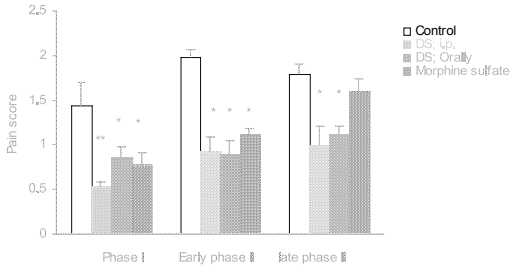

Figure 4. Comparison of the analgesic effect of the DS seed extract and morphine sulfate (as the positive control test), with the control group in the formalin pain. Bars show the mean±SEM of pain score. As demonstrated, administration of the extract vis both i.p. and oral routes (similar to morphine sulfate) diminished the acute phase of formalin pain. However, the chronic formalin phase and especially its late period were significantly attenuated by the extract. n=12, *p<0.05, **p<0.01.

mediated through central nervous system and peripheral mechanisms respectively (18, 19). It is therefore concluded that the extract could alleviate the pain through both central and peripheral mechanisms. However, since the analgesic effect of the extract in i.p and oral routes was nearly similar and dose-dependent, it may therefore be concluded that the DS seed's effective component could pass through the gastrointestinal, successfully. Moreover, the comparison of the analgesic effect of DS seed extract with morphine sulfate as positive control test, revealed that in spite of antinociceptive effect of morphine sulfate on acute pain (hot plate and phase I formalin test) which is consistent with other reports (20-22), it could not exerted a potent analgesic effect in chronic pain. In contrast, the chronic phase of formalin pain and especially its ultimatee periods could be significantly attenuated by DS seed extract. Considering the inflammatory origins of phase II of formalin pain which occurs through release of the local mediators like prostaglandins, kinnins, interleukins, substance p and potassium (19), it can be concluded that this herb may exert some modulatory effect on above-mentioned inflammatory mediators.

In addition, it is concluded that the alcoholic DS seed extract could markedly diminish the acute and chronic pain in formalin and hot plate tests. However, the effective components could pass through the gastrointestinal system without major changes, and considering the high distance between LD50 (2300 mg/kg) and

Table 2. Percent mortality of the rats in different doses of DS seeds extract.

DS (mg/kg, i.p.)	Mortality of the rats (%)	*n*
500	0	12
1000	0	12
1500	10	12
2000	35	14
2500	55	16
3000	90	16
4000	100	14

As indicated, the animals were alive in doses of 500 and 1000 mg/kg. A moderate mortality was observed in 1500 and 2000 mg/kg of the extract, and nearly, 90-100% of the rats were died in doses 3000 and 4000 mg/kg, respectively. However, according to the mentioned results dosage of 2500 mg/kg is closed to the LD_{50}. *n*=14-16, in each dosage.

ED5o, this herb can be introduced a safe analgesic medicinal plant. However, further experiments are needed for isolation and purification of antinociceptive components.

Acknowledgements

We wish to thank Mohsen Ansari from Department of Pharmacology, School of Medicine, Shahed University, for the preparation of the absolute *Datura* seeds extract.

References

(1) Zargari A. *Medicinal Plants.* 1st ed. Tehran University Press (1989) 637-639

(2) Schulman M L and Bolton L A. Datura seed intoxification in two horses. *J. S. Afr. Vet. Assoc.*, (1998) 69: 27-9

(3) Abena A A, Miguel L M, Mouanga A and Hondi Assah T. Evaluation of analgesic effect of *Datura fastuosa* leaves and seed extracts. *Fitoterapia* (2003) 486-488.

(4) Arouko H, Matray M D, Braganca C and Mpaka J P. Voluntary poisoning by ingestion of Datura stramonium. Another cause of hospitalization in youth seeking strong sensation. *Ann. Med. Interne.* (2003) 46-50

(5) Hasan S S and Kushwaha A K. Chronic effect of datura (seed) extract on the brain of albino rats. *Jpn. J. Pharmacol.* (1987) 44: 1-6

(6) Piva G and Piva A. Anti-nutritional factors of Datura in feedstuffs. *Plant toxin.* (1995) 4: 238-41

(7) Berkov S. Alkaloids of *Datura ceratocaula. Z. Naturforsch.* (2003) 455-458

(8) Hartvig P, Gillberg PG and Gordh T. Cholinergic mechanisms in pain and analgesia. *Pharmacol. Sci.* (1989) 75-79

(9) Xu G, Duanmu Z and Yin Q. The role of Ach in the central nerve system on pain modulation and analgesia. *Zhenci Yan Jiu.* (1993) 18: 1-5

(10) Lewis JW, Cannon JT and Liebeskind JC. Involvement of central muscarinic cholinergic

mechanisms in opiate stress analgesia. (1983) 270: 289-93

(11) Pert A and Maxey G. Asymmetrical cross-tolerance between morphine and scopolamine induced antinociception in the primate: differential sites of action. Psychopharmacologia (1975) 44: 139-45

(12) Thor K B, Muhlhuauser M A and Sauerberg P. Central muscarinic inhibition of lower urinry tract nociception. (2000) 870: 124-34

(13) Heidari M R, Khalili M, Hashemi B and Zarrindast M R. Effect of picriotoxine on antinociception in the formalin test. *Pharmacol. Toxicol.* (1996) 78: 313-316

(14) Miller R L, Insel P A and Melnon L K. *Inflammatory disorders. Clinical Pharmacology.* 2nd ed. McMillan, New York (1978) 657-708

(15) Eisner T. Chemical prospecting: A call for action. In: Borman F H and Kellert S R (eds.) *Economic and Ethics: The Broken Circle.* Yale University Press (1990)

(16) Farnsworth NR. Screening plants for new medicines. In: Wilson E O.(ed.) *Biodiversity, Part II.* National Academy Press. Washington, (1989) 83-97

(17) Chevallier A. *The Encyclopedia of Medicinal Plants.* Dorling Kindersley Book, London (1996) 171-179

(18) Shibata M, Ohkubo T, Takahashi H and Inoki R. Modified formalin test: characteristic biphasic pain response. *Pain* (1989) 38: 347-352

(19) Hunskaar S and Hole K. The formalin test in mice:dissociation between inflammatory and non-inflammatory pain. *Pain* (1987) 30: 103-114

(20) Rosland J H, Tjoisen A, Maehle B and Hole H. The formalin test in mice. effect of formalin concentration. *Pain* (1990) 42: 235-242

(21) Yuh-fung C, Huei-yann T and Tian-shung W. Anti-inflammatory and analgestic activities from roots of *Angelica pubescens. Plant Medica* (1994) 61: 2-8

(22) Tjolsen A, Berge O G, Hunskaar S, Rosland J H and Hole K. The formalin test: an evaluation of the method. Pain (1992) 51: 5-17

This article is available online at http://www.ijpr-online.com